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## Examining the potential clinical value of curcumin in the prevention and diagnosis of Alzheimer's disease

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### Abstract

Curcumin derived from turmeric is well documented for its anti-carcinogenic, antioxidant and anti-inflammatory properties. Recent studies show that curcumin also possesses neuroprotective and cognitive-enhancing properties that may help delay or prevent neurodegenerative diseases, including Alzheimer's disease (AD). Currently, clinical diagnosis of AD is onerous, and it is primarily based on the exclusion of other causes of dementia. In addition, phase III clinical trials of potential treatments have mostly failed, leaving disease-modifying interventions elusive. AD can be characterised neuropathologically by the deposition of extracellular  $\beta$  amyloid ( $A\beta$ ) plaques and intracellular accumulation of tau-containing neurofibrillary tangles. Disruptions in  $A\beta$  metabolism/clearance contribute to AD pathogenesis. *In vitro* studies have shown that  $A\beta$  metabolism is altered by curcumin, and animal studies report that curcumin may influence brain function and the development of dementia, because of its antioxidant and anti-inflammatory properties, as well as its ability to influence  $A\beta$  metabolism. However, clinical studies of curcumin have revealed limited effects to date, most likely because of curcumin's relatively low solubility and bioavailability, and because of selection of cohorts with diagnosed AD, in whom there is already major neuropathology. However, the fresh approach of targeting early AD pathology (by treating healthy, pre-clinical and mild cognitive impairment-stage cohorts) combined with new curcumin formulations that increase bioavailability is renewing optimism concerning curcumin-based therapy. The aim of this paper is to review the current evidence supporting an association between curcumin and modulation of AD pathology, including *in vitro* and *in vivo* studies. We also review the use of curcumin in emerging retinal imaging technology, as a fluorochrome for AD diagnostics.

**Key words:** Curcumin: Alzheimer's disease: Amyloid: Retinal imaging

With the ageing of many populations worldwide, it is predicted that over the next few decades there will be a marked increase in the number of people with dementia. Current estimations show that 35.6 million people worldwide have dementia, which is predicted to more than triple to 115 million by 2050<sup>(1)</sup>. Of all the dementia sub-types, Alzheimer's disease (AD) is the most common. AD is a neurodegenerative disease, which is characterised clinically by the progressive loss of memory and cognitive functioning. Major pathological features of an AD brain include the accumulation of extracellular plaques and fibrils, intracellular neurofibrillary tangles (NFT), as well as chronic inflammation and widespread synaptic and neuronal loss, leading to brain atrophy and dysfunction. The deposition

of amyloid plaques is suggested as a defining feature of the AD brain, as NFT are featured in other neurodegenerative diseases<sup>(2,3)</sup> (although plaques have also been reported in cases of non-AD dementias). Nevertheless, hyper-phosphorylated tau protein, the major component of NFT, may have a critical role in the progression of AD, as it acts together with the major protein component of amyloid plaques,  $\beta$  amyloid ( $A\beta$  peptides), driving neurodegeneration<sup>(4,5)</sup>. The  $A\beta$  peptide is generated from its parent molecule, amyloid precursor protein (APP), via sequential proteolytic processing by the enzymes  $\beta$ -APP-cleaving enzyme-1 (BACE1) and  $\gamma$ -secretase<sup>(6)</sup>, to generate multiple  $A\beta$  forms of varying amino acid lengths.  $A\beta$  peptides aggregate readily into oligomers and fibrils, and

**Abbreviations:** AD, Alzheimer's disease;  $A\beta$ ,  $\beta$  amyloid; APP, amyloid precursor protein; BACE1,  $\beta$ -APP-cleaving enzyme-1; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; NFT, neurofibrillary tangles; PSD-95, post-synaptic density 95.

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small oligomers of the longer, more easily aggregating 42-amino-acid form ( $A\beta$ 1-42) are considered to be the most neurotoxic  $A\beta$  species in the AD brain. Amyloid deposition is thought to occur early in the disease process<sup>(7)</sup>, and the accumulation of small  $A\beta$  aggregates ('oligomers') is thought to have a critical role in early pathogenic events that include tau hyperphosphorylation and accumulation, oxidative stress and inflammatory processes that lead to neurodegeneration in the AD brain<sup>(4,8,9)</sup>.

With no current effective disease-modifying treatments available, finding pharmacological/non-pharmacological strategies to halt or slow disease progression is of significant importance. The failure of potential pharmaceuticals in human clinical trials has highlighted the need for research into early diagnosis of AD. This is because of the considerable synaptic loss, neuronal loss and brain shrinkage already present by the time AD clinical symptoms emerge, with treatments aimed at slowing the progress of the disease more likely to be effective before onset of symptoms, preferably at the earliest pre-clinical stage. The continuing lack of effective pharmaceutical drugs has also prompted the evaluation of alternative therapeutics, such as nutraceuticals. Curcumin is one example where, because of its properties as an anti-inflammatory, antioxidant,  $A\beta$ -lowering agent and  $A\beta$  aggregation inhibitor, it shows potential as a therapeutic for AD. In addition, because of its ability to fluoresce and bind  $A\beta$ , curcumin has potential as an imaging agent for diagnostics. This review outlines *in vitro*, *in vivo* and human studies that have evaluated the therapeutic potential of curcumin in AD, and it discusses recent research that has assessed curcumin as a diagnostic tool through its use in emerging retinal imaging technologies. All human studies identified in this review met current National Institute of Health and the Alzheimer's Association diagnostic guidelines<sup>(10)</sup>.

### Beneficial properties of curcumin – historical perspective

Curcumin is extracted from turmeric, a spice that is derived from the rhizomes of *Curcuma Longa* and which belongs to the Zingiberaceae (ginger) family. Turmeric is a perennial herb, native to the monsoon forests of south-east Asia, and it is commonly used in Indian, Asian and Middle Eastern foods. In addition to being used as a culinary spice, turmeric (Sanskrit Haridra, meaning that which is yellow) has been a frequently prescribed herbal medicine. Reputed for its blood-purifying abilities<sup>(11–13)</sup>, Ayurveda medicine and traditional Persian and Chinese medicine have prescribed curcumin for centuries for its body-cleansing properties, as well as for pain associated with inflammation of the skin and muscles. Curcumin has also been prescribed for asthma, bronchial hyperreactivity, allergy, anorexia, coryza, cough, sinusitis and hepatic disease<sup>(14)</sup>.

Only 3–5% of turmeric comprises the yellow-pigmented chemically active curcuminoids, being curcumin (diferuloyl-methane), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC)<sup>(15)</sup>. Curcumin, considered the most therapeutic of the three curcuminoids, was first isolated in 1815 by Vogel and Pelletier<sup>(16)</sup>, although its chemical structure was not confirmed until almost a century later<sup>(17)</sup>.

The twenty-first century has witnessed renewed interest in curcumin's reputed therapeutic effects, which has resulted in

considerable scientific enquiry and review<sup>(13,16,18–21)</sup>. Cell studies<sup>(22–24)</sup> report curcumin to possess powerful anti-inflammatory properties, whereas further research in a variety of inflammatory conditions demonstrates its potential. For example, animal and cell culture studies show that curcumin reduces inflammation in arthritis<sup>(25,26)</sup>; human cell line studies show that curcumin is effective in the management of irritable bowel syndrome<sup>(27)</sup> and in human clinical trials for psoriasis and other skin disorders<sup>(28,29)</sup>. Anti-proliferative and anti-angiogenic influences of curcumin have also been demonstrated, and its therapeutic benefits are shown in human cancer cell and tissue culture, including prostate<sup>(30)</sup>, breast<sup>(31)</sup>, pancreatic<sup>(32)</sup> and bowel cancer<sup>(33)</sup>, as well as head and neck squamous cell carcinoma<sup>(34)</sup>.

Curcumin is also considered a powerful antioxidant, reported to be several times more potent than vitamin E as a free-radical scavenger<sup>(35)</sup>. Curcumin's anti-inflammatory and antioxidant properties have more recently been investigated with respect to AD, as it is now well established that oxidative stress<sup>(36)</sup> and chronic inflammation are central in the early pathogenic stages of AD<sup>(37)</sup>. However, in addition to curcumin altering AD development through anti-inflammatory and antioxidant properties, curcumin's ability to bind to  $A\beta$ , influence deposition and aggregation, while possibly also modulating tau processing, has attracted considerable interest in AD research laboratories<sup>(38–41)</sup>.

Extracellular  $A\beta$  plaques and intraneuronal hyperphosphorylated tau are recognised as hallmark neuropathological features of AD<sup>(42–44)</sup> in addition to oxidative stress and inflammation, and it is believed that abnormal  $A\beta$  metabolism, resulting in high levels of toxic  $A\beta$  oligomers, combined with oxidative stress and inflammation form an AD pathogenic cycle of neurodegeneration. While the initiating step of this neurodegeneration remains to be elucidated, these changes are thought to begin decades before clinical diagnosis; in fact, the accumulation of  $A\beta$  has been shown in radiological imaging to start 20 years or more before the first clinical signs of AD<sup>(45)</sup>.  $A\beta$  accumulation is reported to be associated with impaired synaptic function<sup>(46)</sup>, reduced neurite outgrowth<sup>(47)</sup>, cerebral atrophy<sup>(48,49)</sup> and reduced cognitive performance, particularly when deposited within the temporal region<sup>(50)</sup>. Synaptic/neuronal loss and NFT load have been shown to correlate positively with cerebral atrophy and cognitive decline, whereas cerebral  $A\beta$  load also correlates with cognitive decline, although to a lesser extent<sup>(7)</sup>. However, there is also a large body of evidence suggesting that small oligomers of  $A\beta$  are particularly toxic to neurons, causing membrane damage,  $Ca^{2+}$  leakage, oxidative damage, disruptions to insulin signalling pathways and synaptic function, as well as mitochondrial damage<sup>(51–53)</sup>. As mentioned above,  $A\beta$ -induced changes are believed to occur early in the disease process, and the findings indicate that interventions that can interrupt the production of  $A\beta$  or  $A\beta$  oligomers, or facilitate their removal from the central nervous system, are highly desirable. Modelled projections suggest that delaying the onset of dementia by even 1 year may reduce the worldwide burden of cases in people over 60 years by as much as approximately 10%<sup>(54)</sup>, whereas the introduction of an intervention that delays the onset of dementia by 5 years could reduce the incidence by almost half<sup>(55,56)</sup>. Therefore, early pre-clinical prevention therapy, which could influence the accumulation or clearance of cerebral  $A\beta$  and tau pathology, and/or

reduce oxidative stress and chronic inflammation, thus slowing or reversing these pathological changes, would be highly significant for the reduction of AD prevalence.

### Potentially neuroprotective properties of curcumin: animal studies and *in vitro* anti- $\beta$ amyloid activity of curcumin prevents $\beta$ amyloid aggregation

The  $A\beta$  peptide aggregates readily, first into small aggregates of  $A\beta$  known as  $A\beta$  oligomers and then these oligomers aggregate further to form fibrils, larger fibrils and ultimately plaques of  $A\beta$ . Although plaques and large fibrils are the easiest to detect immunohistochemically, these are considered to be relatively inert: as mentioned above, there is now considerable evidence that small  $A\beta$  oligomers are the main neurotoxic species<sup>(57)</sup>. Therefore, it is interesting that substantial data from *in vitro* studies indicate that curcumin can bind to  $A\beta$  and influence its aggregation. For example, curcumin has been shown to inhibit fibril formation and extension, as well as to destabilise pre-formed fibrils in a dose-dependent manner, effective at concentrations about 0.1–1.0  $\mu\text{M}$ <sup>(58)</sup>. Later studies have similarly shown that curcumin can inhibit the formation of small  $A\beta$  aggregates ( $A\beta$  oligomers) in a dose-dependent manner<sup>(59,60)</sup>.

Studies have investigated how curcumin influences  $A\beta$  aggregation, and different theories have emerged – for example, one theory involves curcumin binding to metal ions. Biomaterials such as Cu (Cu(II)) and Zn (Zn(II)) are found in abundance in the brain, particularly at synapses. Dysregulation of metal homeostasis can lead to the binding of these particular metal ions to  $A\beta$ , and many studies have shown that this binding accelerates  $A\beta$  aggregation. In fact, elevated levels of certain metal ions have been associated with AD<sup>(61)</sup>, and considerable research has been undertaken to understand the normal roles of these ions in the brain, as well as the roles the ions may have in disease pathogenesis, particularly the roles of Cu(II) and Zn(II) on  $A\beta$  aggregation<sup>(62)</sup>. Some recent studies have suggested that curcumin complexes with Cu(II) and/or Zn(II) and that this inhibits the transition from less structured oligomer to  $\beta$ -sheet-rich  $A\beta$  protofibrils, which in turn act as seeding factors for further  $A\beta$  fibrillisation<sup>(63)</sup>. Recent studies looking at the effect of curcumin and curcumin derivatives on metal-induced  $A\beta$  aggregation have shown that Gd-linked curcumin (Gd-Cur, a potential  $A\beta$  imaging agent), compared with curcumin and Cur-S, a water-soluble form of curcumin, could modulate Cu-induced  $A\beta$  aggregation to a greater extent<sup>(64)</sup>, supporting the concept of therapeutic and diagnostic uses for the Gd-Cur compounds.

Other theories do not involve metal ions; instead, they suggest that curcumin's ability to bind  $A\beta$  and inhibit its aggregation is because of curcumin's three structural features: a hydroxyl substitution on the aromatic end group, a rigid linker region between 8 and 16 Å in length and a second terminal phenyl group<sup>(59)</sup>. More recent studies using atomic force microscopy have found that curcumin (and another small-molecule inhibitor resveratrol) binds to the N terminus (residues 5–20) of  $A\beta$ 1–42 monomers and prevents oligomers of 1–2 nm in size from becoming larger 3–5 nm oligomers<sup>(65)</sup>. Yet another recent study has used NMR spectroscopy to investigate the structural modifications of  $A\beta$ 1–42 aggregates induced by curcumin, and

found that curcumin induces major structural changes in the Asp-23–Lys-28 salt bridge region and near the  $A\beta$ 1–42 C terminus<sup>(66)</sup>. The study also used electron microscopy to show that the  $A\beta$ 1–42 fibrils are disrupted by curcumin. Interestingly, in a *Drosophila* AD model, curcumin-fed flies showed accelerated conversion of pre-fibrillar to fibrillar  $A\beta$ , thereby reducing the neurotoxicity of pre-fibrillar  $A\beta$ <sup>(67)</sup>. Overall, curcumin effects are not limited to modulation of  $A\beta$  aggregation, and further studies are needed to determine which effect(s) are the most relevant in promoting brain health in pathological cognitive decline.

### Curcumin influences $\beta$ amyloid production

*In vitro* studies have shown that  $A\beta$  production is influenced by curcumin, as curcumin has been found to inhibit the production of  $A\beta$  peptides by altering APP trafficking through the secretory pathway<sup>(24)</sup>. Zhang *et al.* treated mouse primary cortical neurons with different concentrations of curcumin (1–20  $\mu\text{M}$ ) for 24 h and found that both  $A\beta$ 1–40 and  $A\beta$ 1–42 levels significantly decreased compared with controls. It was suggested that curcumin could stabilise an immature form of APP and reduce the amount reaching the cell surface, thus being available for endocytosis – a process necessary for  $A\beta$  production. In an APP-transfected human embryonic kidney cell culture model (SwAPP HEK293), BDMC was shown to reduce *BACE1* messenger RNA (mRNA) and protein levels, whereas DMC only affected *BACE1* mRNA expression<sup>(68)</sup>. Furthermore, in other studies using a neuronal cell line (pheochromocytoma cells – the PC12 cell line) 3–30  $\mu\text{M}$  curcumin suppressed  $A\beta$ -induced *BACE1* up-regulation<sup>(69,70)</sup>. Most recently, in studies of an AD *Drosophila* model, it was found that the curcumin component BDMC was the most effective at rescuing the flies from the morphological and behavioural defects caused by the overexpression of APP and *BACE1*<sup>(71)</sup>, possibly via inhibition of the *BACE1* enzyme. Recognising curcumin's ability to reduce  $A\beta$  production, by reducing *BACE1* mRNA and its corresponding protein<sup>(72)</sup>, curcumin has been used as a potent positive control in the analysis of other compounds/drugs that target not only *BACE1* but also metal chelation,  $A\beta$  aggregation and oxidation<sup>(73)</sup>. In support of curcumin's metal chelation properties, curcumin was shown to prevent the up-regulation of *APP* and *BACE1* induced by supraphysiological levels of the metal ions Cu(II) and Mn<sup>(74)</sup>.

### Curcumin can inhibit $\beta$ amyloid-induced toxicity

Previous studies support the notion that curcumin can reduce  $A\beta$ -induced toxicity. A study by Park *et al.*<sup>(75)</sup> used PC12 cell cultures pre-treated with 10  $\mu\text{g/ml}$  curcumin before  $A\beta$  exposure. Compared with controls, pre-treated cells had a significant reduction in oxidative stress, as well as lower Ca influx, resulting in protection against DNA damage and cell death. Curcumin (1–30  $\mu\text{M}$ ) has also been shown to attenuate the production of  $A\beta$ -induced radical  $\text{O}_2$  species in neuronal cell cultures, and 20  $\mu\text{M}$  curcumin has been shown to prevent structural changes in  $A\beta$  towards  $\beta$ -sheet-rich secondary structures<sup>(69)</sup>. Furthermore, the protection curcumin provided to PC12 cells and to human umbilical vein endothelial cells against  $A\beta$ 1–42-induced injury was attributed by Kim *et al.*<sup>(76)</sup> to antioxidant mechanisms of

curcuminoids. More recently, *in vitro* studies of microglia have shown that curcumin can dampen inflammatory pathways that promote neurodegeneration<sup>(77)</sup>. In this study, curcumin dose-dependently improved viability against A $\beta$ -42-induced inflammation, as it abolishes A $\beta$ -42-induced IL-1 $\beta$ , IL-6 and TNF- $\alpha$  production. Curcumin was also shown to reduce ERK1/2 and p38 phosphorylation, which was then shown to reduce cytokine production by the microglia<sup>(77)</sup>.

Curcumin's neuroprotective properties may also be attributed to its role in cell signalling. Cell signalling by the Wnt pathways, via the transcription co-activator  $\beta$ -catenin, controls embryonic development, cellular proliferation and neurogenesis. Disruptions to this pathway have been shown to have a significant role in the pathogenesis of diverse diseases such as cancer, metabolic diseases, osteoporosis, epilepsy, as well as AD. In studies of APP-transfected neuroblastoma cells, curcumin was found to activate the Wnt/ $\beta$ -catenin signalling pathway by inhibiting the activity of glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ )<sup>(78)</sup>. GSK-3 $\beta$  is a negative regulator of Wnt, and thus lowering its activity will activate Wnt. However, just as importantly, constitutively active GSK-3 $\beta$  contributes to aberrant tau phosphorylation and NFT formation, which are hallmark pathological changes in AD<sup>(79)</sup>, and thus curcumin-induced inhibition of GSK-3 $\beta$  may also reduce NFT formation. However, the benefits of curcumin in attenuating tau phosphorylation and accumulation have yet to be investigated thoroughly. Interestingly, A $\beta$  oligomers have also been shown to inactivate Wnt in hippocampal slices, by inducing the Wnt antagonist Dickkopf-1<sup>(80)</sup>. These studies collectively suggest that curcumin can influence GSK-3 $\beta$  and Wnt/ $\beta$ -catenin signalling, which are both key factors in AD pathogenesis<sup>(81)</sup>. Furthermore, it has been shown recently that activation of the Wnt/ $\beta$ -catenin signalling pathway inhibits the transcription of BACE1 by binding of T-cell factor-4 to the BACE1 promoter gene, thereby reducing the generation of A $\beta$ <sup>(82)</sup>. Other recent studies using molecular modelling software programs have identified curcumin and rosmarinic acid as promising ligands that mimic the inhibitory action of peptidyl inhibitors of caspase-3<sup>(83)</sup>. The relationships between AD-related proteins and pathways discussed above provide further indication of curcumin's therapeutic potential for the prevention of AD.

### Curcumin and the clearance of $\beta$ amyloid

One anti-AD therapeutic approach involves enhancing the clearance of A $\beta$  from the brain. Several mechanisms have been proposed to assist normal clearance of A $\beta$  from the brain, such as enhancing receptor- or apo-mediated transport across the blood-brain barrier (BBB), efflux of A $\beta$  from the brain being the basis of the peripheral sink hypothesis, targeted immune responses to A $\beta$ , dissolution of amyloid fibrils and microglial activation resulting in phagocytosis of amyloid plaques, as reviewed by Bates *et al.*<sup>(84)</sup>. The significance of the innate immune system in A $\beta$  clearance remains pertinent to intervention and treatment modalities. Although previous attempts to use vaccines to augment the immune response were halted because of the incidence of sterile encephalitis<sup>(85)</sup>, interest in this area remains strong. Macrophage activity and phagocytosis of A $\beta$  has been reported to be deficient in AD, suggesting a contributory factor to

A $\beta$  accumulation<sup>(86)</sup>. Furthermore, a later study that pre-treated the AD macrophages with curcuminoids resulted in increased A $\beta$  uptake in 50% of the macrophages<sup>(87)</sup>.

Curcumin's ability to reduce oxidative damage and amyloid pathology in AD transgenic mice, demonstrated by Garcia-Alloza *et al.*<sup>(40)</sup>, also suggests that curcumin can influence amyloid-induced cytopathology, or macrophage processing of amyloid. Garcia-Alloza *et al.* used multi-photon and *in vivo* imaging to reveal a marked amyloid clearance effect, with 30% plaque size reduction and slowed plaque development, in animals receiving curcumin for 7 d via intravenous tail injections. Fiala *et al.*<sup>(86)</sup> examined curcumin's effect on enhancing phagocytosis of A $\beta$  at a molecular level, and found that curcumin restored the normal A $\beta$ -induced up-regulation of the transcription of  $\beta$ -1,4-mannosyl-glycoprotein 4 $\beta$ -N-acetylglucosaminyltransferase (MGAT3), an enzyme thought to be involved in phagocytosis. Other proteins such as toll-like receptors were also up-regulated. These results indicate that curcumin may correct immune defects in AD patients, suggesting a novel approach to AD immunotherapy<sup>(88)</sup>. In more recent studies by the same group, it was found that 1 $\alpha$ ,25(OH)<sub>2</sub>-vitamin D<sub>3</sub> (1,25D<sub>3</sub>) could restore the defective A $\beta$  phagocytosis in AD macrophages, and that a nuclear vitamin D receptor antagonist could block this phagocytosis. All phagocytes seemed to respond to 1,25D<sub>3</sub>, yet only a subset responded to curcuminoids by up-regulating MGAT3. Nevertheless, in those who did respond, further studies demonstrated that the 1,25D<sub>3</sub> bound to a pocket of the vitamin D<sub>3</sub> receptor that influences genomic events, and curcuminoids bound to a non-genomic pocket<sup>(89,90)</sup>, produced an additive effect.

### Curcumin effects on lipid metabolism

Early research<sup>(91–93)</sup> reported that curcumin had cholesterol-lowering ability, supported by Peschel *et al.*<sup>(94)</sup> who reported that curcumin has a hypocholesterolaemic effect, based on its effect on hepatic gene expression. Feng *et al.*<sup>(95)</sup> also found curcumin to lower cholesterol levels through suppression of Niemann Pick C1-like 1 protein, which is responsible for the uptake of cholesterol through vesicular endocytosis within the intestine. Another potential mechanism for the hypocholesterolaemic effect of curcumin was revealed in studies of rats fed a high-fat diet, in which curcumin was found to decrease significantly the serum levels of TAG, total cholesterol and LDL-cholesterol, when compared with a control group: curcumin was found to up-regulate mRNA levels of cholesterol 7 $\alpha$ -hydroxylase (CYP7A1), a rate-limiting enzyme in the biosynthesis of bile acid from cholesterol<sup>(96)</sup>. More recently, treatment of similar high-fat diet-fed rats with curcumin combined with piperine was found to produce similar changes to the serum lipid profiles of the rats and increased HDL levels, resulting in significant up-regulation of the activities and gene expression of apo A-I, lecithin-cholesterol acyltransferase, CYP7A1 and the LDL receptor<sup>(97)</sup>. As hypercholesterolaemia continues to be considered a likely contributor to AD risk<sup>(98,99)</sup>, the use of curcumin if proven to lower cholesterol could represent another approach, adding to the armoury for AD risk reduction.

### Curcumin and telomerase

Xiao *et al.*<sup>(100)</sup>, investigating the role of telomerase (a ribonuclear protein complex that synthesises and elongates telomeric DNA) in the neuroprotective efficacy of curcumin, found curcumin to be protective against  $A\beta$ -induced oxidative stress and cell toxicity. This neuroprotection was lost when telomerase was inhibited by telomerase RT small interfering RNA, indicating that the neuroprotection provided by curcumin was dependent on the presence of telomerase.

### Focusing on findings in animal studies

Several *in vivo* studies have found that  $A\beta$  deposition and plaque burden in AD-model transgenic mice is decreased following treatment with curcumin<sup>(40,60,101,102)</sup>. Curcumin has also been found to inhibit  $A\beta$ -induced tau phosphorylation<sup>(103)</sup>, to reduce microglial activation, indicating a reduction in inflammation<sup>(102,104)</sup>, and reduced oxidative damage<sup>(104)</sup>. Other studies of transgenic mouse models of AD have shown that curcumin can reduce genomic instability events<sup>(105)</sup>.

In the study by Lim *et al.*<sup>(101)</sup>, AD-model Tg2576 mice aged 10 months old were fed a curcumin diet (160 parts per million (ppm)) for 6 months. The results showed that the curcumin diet significantly lowered the levels of oxidised proteins, the inflammatory cytokine, IL-1 $\beta$ , the astrocyte marker glial fibrillary acidic protein (GFAP), soluble and insoluble  $A\beta$  and also plaque burden. The study found that the reduction in GFAP was localised, such that increased activity was shown in areas around plaques, demonstrating a stimulatory effect of curcumin on the phagocytosis of plaques by microglia. Frautschy *et al.*<sup>(104)</sup>, using Sprague–Dawley rats infused with  $A\beta$ 40 and  $A\beta$ 42 to induce neurodegeneration and  $A\beta$  deposits, found that dietary curcumin (2000 ppm (5.43  $\mu$ mol/g)) suppressed  $A\beta$ -induced oxidative damage and memory impairment, and increased microglial labelling within areas adjacent to  $A\beta$  deposits. They also found that curcumin reversed changes in synaptophysin and post-synaptic density 95 (PSD-95) levels, associated with brain plasticity, as well as improved rat performance in length and latency within the water maze test<sup>(104)</sup>. In similar studies of aged Tg2576 AD-model mice by Yang *et al.*<sup>(60)</sup>, it was demonstrated that curcumin injected peripherally (via the carotid artery) can cross the BBB and bind to amyloid plaques and inhibit the formation of  $A\beta$  oligomers and fibrils<sup>(60)</sup>. Later, Begum *et al.*<sup>(15)</sup> showed similar results and suggested that the dienone bridge present in the chemical structure of curcumin is necessary for this reduction in plaque deposition and the lower protein oxidation observed in the curcumin-treated Tg2576 mice.

More recently, Belviranli *et al.*<sup>(106)</sup> showed that aged female rats supplemented with curcumin for 12 d demonstrated improved spatial memory (Morris water maze test), and their brains exhibited reduced oxidative damage. In other studies using an  $A\beta$ -infused male Sprague–Dawley rat model of AD, the effects of combined curcuminoids, as well as the individual curcumin constituents, were examined in relation to genes related to synaptic plasticity. The genes that were investigated included actin, Ca/calmodulin-dependent protein kinase

type IV, PSD-95 and synaptophysin, and significant effects were noted; for example, a significant increase in synaptophysin expression was found following treatment of the hippocampus with curcuminoids, and both DMC and curcumin were found to increase PSD-95 expression several-fold<sup>(107)</sup>, demonstrating results similar to the earlier rat study carried out by Frautschy *et al.*<sup>(104)</sup>.

### Curcumin and neurogenesis

Curcumin has also been found to stimulate proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus, demonstrating other potentially beneficial effects on neuroplasticity<sup>(108)</sup>. In this study, intraperitoneally administered curcumin activated extracellular signal-regulated kinases (ERK) and p38 kinases, which function in cellular signal transduction pathways that are known to be involved in the regulation of neuronal plasticity and stress responses. More recently, a hybrid compound of curcumin and melatonin (5-(4-hydroxy-phenyl)-3-oxo-pentanoic acid (2-(5-methoxy-1H-indol-3-yl)-ethyl)-amide), known as Z-CM-I-1, developed with the aim of improving neuroprotective properties and BBB permeability, was found to reduce  $A\beta$  accumulation in the hippocampus and cortex of APP/PS1 transgenic mice, and to increase the expression of the synaptic markers synaptophysin and PSD-95, following oral administration at a dose of 50 mg/kg for 3 months<sup>(109)</sup>, encouraging further development of this hybrid compound. APP/PS1 mice were also used in another recent study, which tested the effect of 6 months' dietary supplementation with the curcumin derivative 1,7-bis(4'-hydroxy-3'-trifluoromethoxyphenyl)-4-methoxycarbonyl-ethyl-1,6-heptadiene-3,5-dione (FMeC1). It was found that FMeC1 supplementation resulted in less  $A\beta$  deposits, glial cell activity and cognitive deficits, when compared with untreated, curcumin-treated or FMeC2-treated mice, suggesting that FMeC1 has potential in the treatment of AD<sup>(110)</sup>. In rat studies, genetic transcriptional responses along with enhanced hippocampal neurogenesis was seen, following 12 weeks of administration of a curcumin-containing diet, as compared with 6 weeks of this diet, or a control diet<sup>(111)</sup>, providing further evidence that curcumin may be beneficial through the promotion of neuronal cell growth.

### Curcumin and the blood–brain barrier

Studies of rat and mouse models of ischaemic damage found that curcumin could protect the BBB, most likely because of anti-inflammatory and antioxidant effects<sup>(112,113)</sup>, and later studies of cerebral ischaemia in rats found that a single intravenous injection of curcumin could reduce infarct volume and neurological deficit, possibly because of inhibition of inducible nitric oxide synthase<sup>(114)</sup>. More recent studies suggest that curcumin up-regulates heme oxygenase-1 expression to reduce damage and permeability of the BBB<sup>(115)</sup>. Encouragingly, an *in vivo* rat study, using nanoparticulation of curcumin, was able to demonstrate increased organ, as well as the brain, perfusion by curcumin, by prolonging retention time in the hippocampus by 83% and in the cerebral cortex by 96%<sup>(116)</sup>. More recently, another group produced a highly stable nanocurcumin



formulation (particulate size <80 nm) for use within an *in vitro* and in an AD transgenic mouse model. The study showed higher concentrations of the nanoformulation in plasma and within the brain compared with non-capsulated curcumin or placebo, while demonstrating significant improvements in working and cued memory function<sup>(117)</sup>.

Curcumin analogues with similar biological activity to curcumin, yet with improved pharmacokinetic characteristics, including increased bioavailability and water solubility, are continuing to be developed<sup>(118,119)</sup>, while new synthetic products are also emerging<sup>(119)</sup>. Nanotechnology is particularly promising, whereby nanoencapsulation may be able to achieve a synergistic drug delivery system<sup>(120)</sup>. Encouraging results have already been reported in a study examining curcumin for use in breast cancer chemoprevention, which used injectable polymeric micro particles in mice, achieving both sustained blood curcumin levels for almost a month while maximising its BBB penetration, as well as inhibiting tumour vasculature<sup>(121)</sup>. Other studies exploring nanoparticle technology<sup>(122,123)</sup> have been equally promising. For example, following the intravenous administration of liposomal curcumin, polymeric nanocurcumin and poly-lactic-co-glycolic acid co-polymer-curcumin in rats, all of these compounds were found to cross the BBB<sup>(122)</sup>, whereas in another study nanoparticles containing curcumin were shown to increase oral bioavailability 9-fold<sup>(123)</sup>. Further evidence of BBB penetration has been obtained in animal models using labelled curcumin derivatives<sup>(119)</sup>.

### Curcumin and acetylcholinesterase

In addition to the effects above, curcumin has also been shown to influence acetylcholinesterase activities<sup>(124)</sup>, following the same pathway as the commonly prescribed pharmaceuticals, acetylcholinesterase inhibitors, which are considered first-line management in AD<sup>(18,125)</sup>. The administration of acetylcholinesterase inhibitors has been found in certain circumstances to slow the progression of AD symptoms or even reduce AD symptoms for a 12-month period, by inhibiting the breakdown of acetylcholine, a major neurotransmitter, depleted in the AD brain. Using *in vitro* and *ex vivo* models of acetylcholinesterase activity, Ahmed *et al.*<sup>(107)</sup> investigated whether curcumin had an influence on acetylcholinesterase mechanisms, and recorded dose-dependent inhibitory effects in the frontal cortex and in the hippocampal tissue; curcuminoids also demonstrated significant attenuation of scopolamine-induced amnesia. Furthermore, Ahmed *et al.*<sup>(107)</sup> examined the influence of curcumin on spatial memory in amyloid-infused AD rat models, reporting increased PSD-95 and synaptophysin expression in the hippocampus and a memory-enhancing effect. In studies of streptozotocin-induced diabetic rats, curcumin has been shown to prevent cholinergic-mediated cortical dysfunctions, which are induced by diabetes<sup>(126)</sup>, and in mice treated with okadaic acid to induce memory impairment orally administered curcumin has been found to improve cholinergic function and reduce inflammation, among other beneficial effects<sup>(127)</sup>. Furthermore, curcumin has been shown to reverse alcohol-induced cognitive deficits in the adult rat brain, partly by preventing the alcohol-induced activation of acetylcholinesterase; curcumin also reduces

signs of neuroinflammation in these rats<sup>(128)</sup>. Another rat study indicated that curcumin may inhibit acetylcholinesterase activity in As- and Al-induced toxicity models<sup>(129)</sup>.

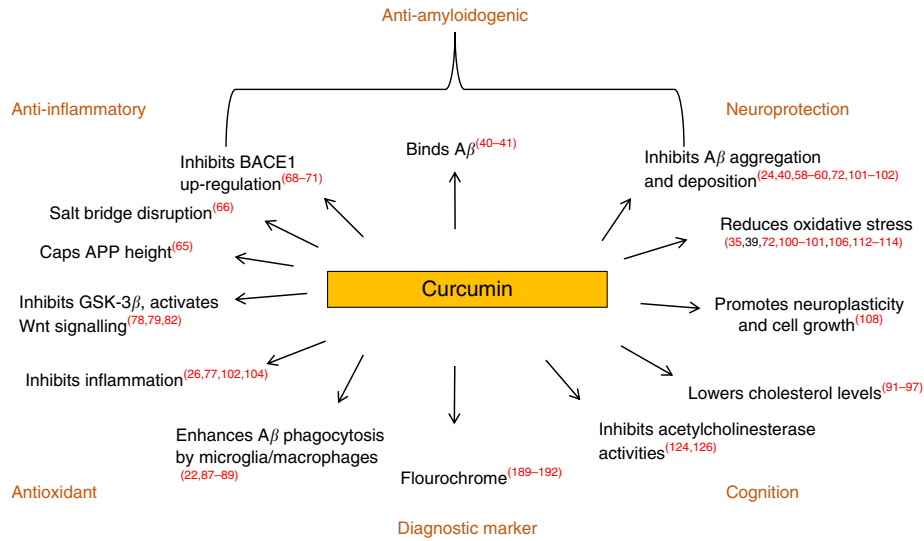
Despite all these animal studies, the influence of curcumin on acetylcholinesterase has not yet been investigated in human clinical studies. Furthermore, mechanisms underlying many of the effects described above are still being characterised. However, there is now evidence of curcumin derivatives influencing proteasomal function and A $\beta$  degradation, as described below.

### Curcumin, proteasome function and $\beta$ amyloid degradation

Proteasomal activity and its role in the degradation of most oxidised proteins is linked with the processes of cell ageing; it is also believed that age-related decreases in proteasome activity weakens a cell's capacity to remove oxidatively modified proteins and therefore encourages the development of diseases<sup>(130)</sup>. Curcumin has been demonstrated to have a stimulatory effect on proteasomal activity, causing a 46% increase in activity at doses of 1  $\mu$ m *in vitro*, whereas higher doses, not likely to be achieved *in vivo*, led to decreased activity<sup>(131)</sup>. More recent studies have shown that a synthetic derivative of curcumin, CNB-001, can stimulate A $\beta$  degradation through both proteasomes and lysosomes, and the experimental inhibition of the proteasome pathway redirects clearance through lysosomes. Other recent CNB-001 studies have provided a link between the findings of several other AD-related biochemical changes. These include the findings that levels of the enzyme 5-lipoxygenase (5-LOX) are elevated in AD<sup>(132)</sup> and that disruption of this enzyme and some phospholipases can reduce AD pathology<sup>(133,134)</sup>; as well as that chronic stress can cause cell signalling/over-activation of regulatory kinases, which in turn leads to the phosphorylation of the eukaryotic initiation factor-2 $\alpha$  (eIF2 $\alpha$ ) and disrupts the translation activation of several mRNA, and detected in neurodegenerative diseases including AD<sup>(135)</sup>. The CNB-001 studies found that CNB-001 could inhibit 5-LOX, which induces the eIF2 $\alpha$  phosphorylation. Furthermore, when fed to AD transgenic mice, CNB-001 was found to increase eIF2 $\alpha$  phosphorylation (as well as heat shock protein 90 and activating transcription factor 4 levels), improve A $\beta$  clearance and therefore limit the accumulation of soluble A $\beta$  and ubiquitinated aggregated proteins. CNB-001 has also been found to maintain the expression of synapse-associated proteins and to improve memory in the mice<sup>(136)</sup>. These studies indicate that the curcuminoid derivative's inhibition of 5-LOX has potential as a therapeutic approach.

Overall, cell culture and animal studies have indicated that curcumin has considerable potential as an inhibitor of A $\beta$  aggregation, as an antioxidant, an anti-inflammatory and as an inhibitor of BACE1. Curcumin, among its modalities of action, has also shown promise in facilitating A $\beta$  clearance/degradation, inhibiting tau phosphorylation, promoting neurogenesis and modulating synaptic plasticity (Fig. 1). Despite these benefits, there is a paucity of population-based studies examining the protective role of curcumin on cognition.





**Fig. 1.** Curcumin: reported mechanisms of action. BACE1,  $\beta$ -APP-cleaving enzyme-1; A $\beta$ ,  $\beta$  amyloid; APP, amyloid precursor protein.

### Effects of curcumin on human cognition

Only a handful of clinical studies have been carried out to evaluate the cognitive enhancing potential of curcumin in AD patients<sup>(137,138)</sup>; however, these have not been particularly successful. Reasons could be because of the low bioavailability of curcumin<sup>(15)</sup>, thereby markedly reducing its potential to reach the brain at sufficient concentrations to provide benefits. Alternatively, the subjects may have been treated at a stage of pathology that is too advanced for curcumin to provide benefits. Nevertheless, there are epidemiological data that support the concept that curcumin can reduce the risk of AD. For example, India, with an estimated average daily consumption of curcumin being 80–200 mg<sup>(21,139)</sup>, has been reported to have a lower incidence and prevalence of AD<sup>(140–142)</sup>, although under-reporting and clinician access may be a contributor. Nevertheless, a study of 1010 cognitively intact Asian participants aged 60–93 years has found that those who consumed curry (which contains turmeric) more often, compared with those who ate curry very rarely or never, performed better on the Mini Mental State Examination (MMSE)<sup>(143)</sup>. These observations, among others, support the concept that turmeric, and in particular its curcumin particle, may possess valuable neuroprotective or cognitive-enhancing properties. However, as mentioned above, clinical trials examining the efficacy of curcumin in patients with cognitive decline have been disappointing; however, more recently, with studies using improved formulations and more appropriate cohorts, encouraging signs are emerging<sup>(144)</sup>. Table 1 represents a list of ongoing/completed clinical trials that have used curcumin for the diagnosis, prevention or treatment of AD. These trials are discussed further below.

Baum *et al.*<sup>(137)</sup> randomised AD patients (*n* 34) to receive 1 g (plus 3 g placebo), 4 g (plus 3 g placebo) or 0 g of oral curcumin (plus 4 g of placebo), once daily. Participants were given the choice of formulation, being either powder or capsule. The intervention group did not demonstrate significant differences in MMSE scores or plasma A $\beta$ -40 levels between 0 and 6 months; however, it was

suggested that the outcome measures were not sensitive or specific enough to demonstrate effects<sup>(137)</sup>. Ringman *et al.*<sup>(145)</sup> conducted a 24-week, randomised, double-blind, placebo-controlled study evaluating the efficacy of two dosages of curcumin (2 and 4 g/d) in patients with mild-to-moderate AD, with an open-label extension for 48 weeks. This was the first study to include measurement of cerebrospinal fluid (CSF) biomarkers. The preliminary results showed no significant differences in cognitive function, in plasma or CSF A $\beta$ -40/A $\beta$ -42 or tau, between placebo and intervention groups; however, bioavailability was again reported as a limitation, although the dosing was well tolerated.

The above studies included AD-diagnosed participants, in whom significant neurodegeneration and AD pathology already exists. Given that the pathological changes begin two decades or more before the first recognisable symptoms<sup>(45)</sup>, targeting healthy older cohorts or those in the pre-clinical or prodromal AD phase would more likely provide benefits through slowing the pathogenic mechanisms. Considerable synaptic and neuronal loss has already occurred by the time symptoms appear, and the antioxidant, anti-inflammatory and A $\beta$ -lowering and anti-A $\beta$  aggregation properties of curcumin are most likely to be of benefit in the early stages, for the prevention of AD pathogenesis. However, curcumin treatment of AD patients may still provide many benefits, and it warrants further clinical evaluation.

More recent studies have evaluated curcumin's effects under normal physiological conditions. In a placebo-controlled study targeting healthy middle-aged subjects (*n* 38, 40–60 years), 80 mg of curcumin (400 mg of Longvida-optimised curcumin) was given orally for 4 weeks to assess the health-promoting effects of curcumin<sup>(146)</sup>. This study, because of the diverse health claims of curcumin, investigated several blood and saliva biomarkers, to examine the effect of curcumin on markers associated with lipids, inflammation, liver function, immunity and stress, as well as A $\beta$  levels. Cognitive measures were not included in their study design. Statistically significant results were shown for a number of these markers including increased catalase, nitric oxide and antioxidant status, with lowered

**Table 1.** Studies using curcumin in Alzheimer’s disease (AD): diagnosis, prevention and treatment

Study	Agent	Cohort	Dose	Duration	End points and brief summary of results
Baum <i>et al.</i> <sup>(137)</sup> (NCT00164749)	Curcumin and ginkgo	Probable AD; 50 years+; <i>n</i> 30	1, 4 g daily	6 months	Safety and effects, biochemical and cognitive measures No differences detected between treatment groups in Aβ levels or MMSE scores
Ringman <i>et al.</i> <sup>(145)</sup> (ACT00099710)	Curcumin C3 complex <sup>®</sup>	Mild/moderate AD; age 49 years+; <i>n</i> 30	2, 4 g daily	24 weeks plus 24 open label	Side effects, blood biomarkers and cognition No differences detected between treatment groups in clinical or biomarker efficacy measures; results also indicated low bioavailability
Hishikawa <i>et al.</i> <sup>(150)</sup>	Turmeric capsules	Severe AD; <i>n</i> 3	100 mg curcumin daily	12 months, tested after 12 weeks	MMSE and NPIQ; score on NPIQ decreased significantly, MMSE increased in 1/3
Poncha (NCT01001637)	Longvida™	Moderate – severe AD; 50–80 years; <i>n</i> 160	2, 3 g twice daily	2 months	Efficacy and safety; blood and cognition
Martins & Goozee (ACTRN12613000681752)	Biocurcumax™ BCM-95	Retirement living, healthy 65–90 years; <i>n</i> 100	500 mg, thrice daily	12 months	Cognition, blood biomarkers/chemistry; lifestyle questionnaires; brain imaging (MRI, PET FDG and amyloid), retinal imaging
Martins (ACTRN12611000437965)	Biocurcumax™ BCM-95	Community living, healthy 55–75 years; <i>n</i> 100	500 mg, thrice daily	12 months	Cognition, blood biomarkers/chemistry; lifestyle brain imaging
Small (NCT01383161)	Theracurmin CR-031P™	MCI/normal ageing <i>n</i> 132	90 mg/d twice daily	18 months	Cognition; blood; genetic profile
Frautschy (NCT018811381)	Longvida and yoga	Subjective cognitive complainers 55–90 years; <i>n</i> 80	400 mg, twice daily	6 months	Biochemistry, cognition and FDG PET
Cox <i>et al.</i> <sup>(144)</sup> (ACTRN12612001027808)	Longvida™	Healthy and cognitive decline 65–80 years; <i>n</i> 60	400, 800 mg daily	Phase 1: acute 1–3 h/4 weeks Phase 2: 8 weeks	Cognition, mood and anxiety; blood biomarkers fMRI; cognition
Patterson (NCT00595582 early termination)	Curcumin bioperine	MCI 55–85 years; <i>n</i> 10	900 mg twice daily	24 months	Cognition and size of metabolic lesions on the PET scan
Martins & Goozee (ACTRN12614001024639)	Biocurcumax™ BCM-95	Healthy and MCI 65–90 years; <i>n</i> 48	500 mg twice daily	3 months	Gene regulation and expression; and cognition
Verdooner & Martins (ACTRN12613000367741)	Longvida™	Healthy, MCI, mild/moderate AD 50 years+; <i>n</i> 200	20 g daily (shake)	7 d	Diagnostics; curcumin fluorescence retinal imaging of Aβ plaques

Aβ, β amyloid; MMSE, Mini Mental State Examination; PET, positron emission tomography; FDG, fluorodeoxyglucose; MCI, mild cognitive impairment; NPIQ, Neuro-Psychiatric Inventory-Brief Questionnaire; fMRI, functional MRI.

plasma alanine aminotransferase and TAG, but not total cholesterol. In addition, curcumin was found to lower plasma  $A\beta$  levels. Another interesting finding was that salivary amylase was also significantly lowered, which is an enzyme associated with adrenergic activity during stress<sup>(147)</sup>.

A number of studies of curcumin supplementation in healthy older subjects are still in progress. However, one such study has been completed: a randomised, double-blind placebo-controlled study ( $n$  60, 60–85 years) using the same 80 mg/d curcumin formulation as used by DiSilvestro *et al.*<sup>(146)</sup> (400 mg of Longvida-optimised curcumin). The authors reported acute (1 h post dose) and chronic (1-month duration) effects of curcumin intake on cognition, mood and blood biomarkers<sup>(144)</sup>. Benefits on attention and working memory were reported following the acute administration of curcumin, whereas at the 1-month time point, working memory and mood improved. Alertness and contentedness also improved after acute-on-chronic treatment.

Although the results above are encouraging, alternate mechanisms, including modulation of the stress response, may have played a part. Amylase, shown to be lowered in an earlier study using curcumin<sup>(146)</sup>, is a recognised biomarker of  $\beta$ -adrenergic stimulation<sup>(147–149)</sup>, and improved attention, working memory and contentedness may be linked to this mode of action. No alterations in blood levels of  $A\beta$ -40 or  $A\beta$ -42 levels were detected, although these are not thought to be reliable biomarkers on their own. Differences in cognitive performance, as demonstrated in serial 7-s and delayed recall, were not significant<sup>(144)</sup>. Nevertheless, curcumin did enhance the lipid profile by lowering LDL, and in contrast to the results found by DiSilvestro *et al.*<sup>(146)</sup>, Cox *et al.*<sup>(144)</sup> also recorded a reduction in plasma total cholesterol. Long-term lipid changes such as these may have some effect on AD risk: as mentioned earlier, chronic conditions linked to abnormal lipid profiles such as obesity and diabetes are linked with a higher risk of AD. Interestingly, a case study reported by Hishikawa *et al.*<sup>(150)</sup> found that three severe-stage AD patients treated with 100 mg/d oral curcumin for 12 weeks (in addition to their already prescribed acetylcholinesterase inhibitor, donepezil) showed a reduction in agitation, anxiety and irritability; one patient also showed improvement in MMSE score. This may suggest a role for curcumin as a concurrent intervention, and it supports the concept that curcumin may provide benefits, even in advanced stages of AD; however, further research would be required to support these suggestions.

As mentioned earlier, several other clinical studies are still underway, or results have not yet been published; thus, with so few clinical studies having been completed, it is not possible to make any conclusions concerning the clinical significance of curcumin in enhancing cognition.

### Epigenetics, Alzheimer's disease and curcumin

Epigenetic alterations have been reported to occur in AD<sup>(151–154)</sup>. As epigenetic alterations are dynamic, these alterations have been proposed as a target area for AD prevention. Epigenetic alterations include changes in DNA methylation, histone modifications or changes in miRNA expression. Studies including some clinical

studies of other conditions have shown that curcumin has the potential to induce epigenetic changes<sup>(155,156)</sup>. For example, epigenetic effects of curcumin have been shown in patients with breast cancer and advanced pancreatic cancer, and also in people at risk of stroke, by providing vascular protection<sup>(157)</sup>. Curcumin has been shown to inhibit DNA methyltransferase, histone acetyltransferase and histone deacetylase, and to modulate miRNA, for example down-regulating microRNA-134 and microRNA-124 in cultured hippocampal slices, which are associated with an increase in the brain-derived neurotrophic factor (BDNF)<sup>(158)</sup>. BDNF has been shown to increase hippocampal neuronal survival and to enhance synaptic plasticity. Furthermore, variants of the BDNF gene have been linked to several mental disorders such as major depressive disorder (MDD) and schizophrenia, and low levels of BDNF protein are thought to contribute to the pathology of MDD. Interestingly, antidepressants have also been found to increase blood levels of BDNF<sup>(159)</sup>. Depression is a major risk factor for AD, and thus this BDNF-modifying property of curcumin is of significant interest. Other recent studies have also found that curcumin can have a significant effect on depression<sup>(160)</sup>, supported to some extent by the studies described above by Cox *et al.*<sup>(144)</sup>

Many other studies have discovered the beneficial epigenetic effects of curcumin in relation to various cancers and rheumatoid arthritis, and now similar benefits are being discovered that may have an impact on the risk and severity of AD<sup>(161)</sup>. For example, DNA methylation in neurodegenerative diseases (and many other conditions such as CVD and stroke) has been linked to high homocysteine levels, which occur with ageing and with vitamin B<sub>12</sub> or folate deficiencies<sup>(162,163)</sup>. Chronically high homocysteine levels lead to an abnormally high DNA methylation<sup>(164)</sup>, which requires DNA methyltransferase, and as mentioned above curcumin inhibits DNA methyltransferase. However, rat studies of homocysteine effects suggest that curcumin may be neuroprotective, and may improve learning and memory deficits, by reducing lipid peroxidation and high malondialdehyde levels, both of which are induced by high homocysteine levels<sup>(165)</sup>. The relative significance of these potential benefits of curcumin dietary supplementation are clearly still not known, and thus further clinical studies are required to evaluate the neuroprotective role of curcumin induced by epigenetic regulation for the prevention of cognitive decline.

### Curcumin safety profile, tolerability, bioavailability and mode of administration

As curcumin is a component of the spice turmeric, it is not surprising that curcumin has been reported to be a very safe nutraceutical with a low side-effect profile. However, it should be noted that while curcumin has been reported to be safe and well tolerated at doses of up to 8 g/d<sup>(166)</sup>, studies have not gone beyond 3 months, and thus the long-term effects of high doses of curcumin are not known. In addition, with enhanced bioavailability and absorption now possible with new formulations, the risk of increased toxicity is higher, particularly for populations taking medications metabolised by the liver or for those with existing liver impairment<sup>(137)</sup>. Nevertheless, although curcumin may not have been tested widely for the purposes of reducing



neurodegeneration, it has been clinically tested in patients with various conditions and pro-inflammatory diseases including cancer, CVD, arthritis, Crohn's disease, ulcerative colitis, irritable bowel disease, tropical pancreatitis, peptic ulcer, psoriasis, atherosclerosis, diabetes, diabetic nephropathy and renal conditions, among others, and has resulted in minimal side effects and many health benefits. Curcumin has also provided protection against hepatic conditions, chronic arsenic exposure and alcohol intoxication<sup>(167)</sup>.

Curcumin's pleiotropic effects explain its wide variety of applications; for example, it has been tested for the purpose of stent coating, as curcumin has advantageous anti-coagulant properties<sup>(168)</sup>. In addition, it can inhibit the generation of blood clotting factors Xa and thrombin via the extrinsic and intrinsic pathways<sup>(169)</sup>. These properties do indicate that one should be cautious when prescribing curcumin in combination with other blood-thinning preparations. Although the safety of curcumin has been demonstrated<sup>(137,145)</sup>, in humans, oral ingestion of existing formulations has presented challenges concerning absorption and bioavailability<sup>(145,170)</sup>. The literature reports that oral curcumin has efficient first-pass metabolism and some degree of intestinal metabolism, including glucuronidation and sulphation (although this occurs mostly in the liver); however, it is excreted largely unconjugated via the intestine. Curcumin is also unstable at neutral and alkaline pH. There appears to be minimal distribution of curcumin to the liver or other tissues beyond the gastrointestinal tract<sup>(171)</sup>. For example, in rat studies, an oral dose of 500 mg/kg resulted in a peak plasma concentration of only 1.8 ng/ml<sup>(172)</sup>, with the major metabolites being curcumin sulphate and curcumin glucuronide, whereas a clinical study found that oral doses of 4, 6 and 8 g of curcumin daily for 3 months yielded serum curcumin concentrations of only 0.51 (SD 0.11), 0.63 (SD 0.06) and 1.77 (SD 1.87)  $\mu\text{M}$ , respectively, with peak levels at 1–2 h post dosing<sup>(166)</sup>. Unmodified curcumin is reported to be retained in the blood for 2–5 h in humans, whereas retention of a modified form of curcumin – Biocurcumin-95 (BCM-95) – is reported as exceeding 8 h<sup>(173)</sup>. In order for the curcumin to elicit a greater nutraceutical benefit, it is critical that more of it is able to enter the bloodstream, it must have a longer half-life and also cross the BBB to be of significant benefit in AD.

To date, most human curcumin studies have used oral formulations. Absorption and bioavailability have continued to be a hindrance, not aided by the variation of formulations available. However, as technology has advanced and new delivery approaches have emerged, the use of adjuvant therapies, isomerisation, liposomes, micelles, phospholipids and nanotechnology also increase. One of the potential therapeutic results of increasing blood levels of curcumin in humans can hopefully be anticipated from AD transgenic mouse studies, in which the intravenous administration of curcumin (7.7 mg/kg per d) for 7 d resulted in significant clearance of cerebral  $A\beta$  load<sup>(40)</sup>. Alternate routes of delivering curcumin are already being used for other disorders, such as the use of topical eye drops, recommended for the treatment of a variety of ophthalmic disorders<sup>(174)</sup>, and transdermal application using encapsulated curcumin as a nanoemulsion, for the treatment of arthritis<sup>(175)</sup>. Recently, the curcumin derivative FMeC1, originally produced as an MRI probe, has been produced in aerosol form for inhalation.

A study in 5XFAD transgenic mice suggested improved distribution in the brain, and immunohistochemical studies demonstrated that FMeC1 absorbed following aerosol delivery did bind to amyloid plaques in the mouse brains<sup>(176)</sup>. This technique may also be useful for  $A\beta$  imaging studies; however, further studies are needed to validate this notion. The absorption and bioavailability of curcumin is highly relevant, and the formulation, dose and mode of delivery are each important factors. Multiple over-the-counter brands are available, and most of them claim increased bioavailability compared with unformulated curcumin; however, independent comparative analysis is essential. Two formulations BCM-95<sup>(177,178)</sup> and Longvida<sup>(145)</sup> currently have the strongest independent data available in human trials. For a review of the molecular structure of curcumin and its derivatives, FMeC1 and FMeC2, see Yanagisawa *et al.*<sup>(110)</sup>, and differences between the properties of CNB-001 can be examined as previously published<sup>(179–181)</sup>.

To summarise, curcumin has been trialled at doses as high as 8 g/d, and found to be well tolerated and safe. However, as new formulations are emerging that are showing promise of increasing bioavailability, BBB permeability and longer half-lives, these formulations also need to be evaluated in future safety and tolerability trials. Furthermore, as any curcumin therapy is likely to be long-term in nature, much longer treatment times need to be trialled. The animal and clinical studies that have investigated the role of curcumin have applied a variety of administration modes, including oral, subcutaneous, intraperitoneal, intravenous, topical and the nasal route<sup>(182)</sup>. Human trials investigating curcumin's neuroprotective mechanisms have mostly used the oral route; however, future studies should explore other routes of administration.

### Curcumin as a fluorochrome/radioligand in Alzheimer's disease diagnosis

Turmeric has been used as a colouring agent since ancient times. In 1989, Stockert *et al.*<sup>(183)</sup> identified curcumin as a potential fluorochrome, as curcumin was found to fluoresce yellow/green under a violet/blue (436 nm) light, and it was noted to bind to DNA and chromosomes, as treatment of tissue samples and cell samples with deoxyribonuclease or TCA prevented the chromatin staining. More recently, these innate fluorescent qualities (curcumin absorbs light at about 420 nm and emits fluorescence at about 530 nm in aqueous solutions<sup>(184)</sup>), and curcumin's natural affinity to bind with  $A\beta$ , prompted curcumin to be tested as a safe plaque-labelling fluorochrome. A mouse study investigated novel derivatives of curcumin and measured their binding affinities for  $A\beta$  aggregates<sup>(185)</sup>. The derivative with the highest affinity was then (<sup>18</sup>F)-radiolabelled for testing as a radioligand probe for  $A\beta$  plaque imaging; the compound also had suitable lipophilicity, good brain uptake and was metabolically stable in the brain. In another study conducted on transgenic AD mice, multiphoton microscopy was used to demonstrate that curcumin crossed the BBB and labelled  $A\beta$  plaques and cerebrovascular amyloid angiopathy<sup>(40)</sup>. Curcumin has since been used in the labelling of neuronal fibrillar tau inclusions in human brain samples of AD and progressive supranuclear palsy<sup>(186)</sup>. More recently, other studies of  $A\beta$  imaging

used an  $^{19}\text{F}$ -containing curcumin derivative injected peripherally in AD-model mice to detect  $\text{A}\beta$  plaques in the brains, using MRI<sup>(110)</sup>. Furthermore, Koronyo-Hamaoui *et al.*<sup>(187)</sup> demonstrated curcumin's value as a staining agent for  $\text{A}\beta$  by detecting plaques in human postmortem retinal tissue, and also as a brain and retinal  $\text{A}\beta$  plaque tracer administered intravenously in transgenic mice. Importantly, the pathology in the retina was detected before the stage at which pathology in the brain could be detected, indicating that curcumin may have potential as a pre-clinical AD biomarker. The research also supports the previous observation that curcumin has the ability to cross the BBB, which is essential for its therapeutic efficacy. Preliminary data from a pilot study ( $n$  40) conducted by our group undertaking retinal imaging using curcumin as a fluorochrome had a 100 % sensitivity and 80.6 % specificity for AD diagnosis<sup>(3)</sup>. Another study recruited mild cognitive impairment (MCI) patients ( $n$  30) and administered 80 mg of curcumin (Meriva) twice daily for 3 d and found abnormal deposits in different retinal layers believed to be related to neurodegeneration<sup>(188)</sup>. The study reported that curcumin caused patchy hypofluorescent spots; however, it did not quantify the retinal amyloid plaques. The findings were primarily based on the direct perception of the deposits via ocular imaging. Recent studies have again used MRI, this time to detect magnetic nanoparticles made of superparamagnetic iron oxide conjugated with curcumin, which were found to bind to  $\text{A}\beta$  aggregates in *ex vivo* AD-model mouse brains, after injection with the curcumin conjugate<sup>(189)</sup>. Other recent studies have produced a novel nanoimaging agent: poly( $\beta$ -l-malic acid) containing covalently attached (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) gadolinium and curcumin. The all-in-one agent selectively binds to  $\text{A}\beta$  plaques and can be detected by MRI<sup>(190)</sup>, thus providing another promising  $\text{A}\beta$  plaque imaging agent.

Curcumin, being non-toxic, accessible and economical, thus becomes highly attractive for both diagnostics and therapeutic research. As discussed above, retinal imaging using curcumin fluorescence is currently being examined in our research centre as part of the Australian Imaging, Biomarkers and Lifestyle flagship study of ageing, and it has been found to be well tolerated by participants. This approach, combined with examination of the retinal vascular features<sup>(191)</sup>, may deliver a novel diagnostic tool that provides a more reliable indication of early AD changes, which is economical, relatively non-invasive and widely applicable.

#### Limitations of curcumin and future directions

If all the positive results observed in *in vitro* and *in vivo* animal studies could be translated into human studies, the significance of curcumin in AD prevention and treatment would be considerable. The recent study by Cox *et al.*<sup>(144)</sup> provided some encouraging outcomes by investigating cognitive markers, but only acute changes in attention, working memory and mood were significant. No effects were reported in long-term memory or executive function; however, the short duration of the study may have been a limitation. The evidence that curcumin can influence  $\text{A}\beta$  aggregation and  $\text{A}\beta$  clearance, support innate immune systems, reduce oxidative stress, enhance cognition and impede the onset of AD in humans remains elusive. However, not to be overlooked is the application of curcumin

as a diagnostic fluorochrome, potentially assisting in the earlier identification of pre-clinical stages of AD, during retinal scanning. The use of curcumin as a fluorochrome within retinal amyloid imaging, combined with examination of retinal vascular features, offers a novel diagnostic approach to AD. While retinal imaging is acknowledged as a non-invasive, economical and easily translated technology, further validation is required before it can be adopted as an early AD marker.

Enhanced oral formulations of curcumin are emerging, potentially negating the prior challenges of absorption and bioavailability. However, considering the differences in product formulation, and the multitude of curcumin products already available, comparative analysis would be useful. As the primary focus of AD treatment has turned to primary prevention, the point of intervention is also crucial: interventions introduced early (>10 years before the onset of AD clinical symptoms) may present difficulty establishing statistically significant changes, whereas interventions introduced 1–2 years before the onset may be less effective or ineffective as the disease pathology may already be too advanced. Prevention studies designed with longer duration are highly desirable; however, as nutraceuticals generally do not attract commercial opportunity, realising these types of studies will be difficult to accomplish.

The use of curcumin as an adjunct therapy to cholinesterase inhibitors, particularly in the early stages of AD, offers a potentially new area of research. As anxiety and stress are common co-morbidities in AD, and research has shown curcumin to have effect in these areas, curcumin may offer an appealing alternative to antidepressant and antipsychotic therapies, while potentially offering other synergies, including influencing the underlying neuropathology and enhancing cholinergic activity. In recent years, the focus on curcumin as a compound of interest for the prevention of AD has been intensifying. The results of ongoing clinical trials will hopefully shed more light on the benefits of curcumin in the prevention of AD. In line with curcumin's complex modes of action, outcome measures should be expanded to include not just cognitive changes; extensive blood biomarker assays should also be carried out, as well as imaging (e.g. measuring cerebral amyloid load and potentially retinal markers) to characterise curcumin's effects more fully over time, in a pre-clinical population.

#### Conclusion

To date, AD clinical trials have not been able to generate the anticipated benefits of curcumin; however, this has been broadly attributed to difficulties with absorption, bioavailability and arguably the timing and length of intervention. As reviewed in this article, there is significant evidence that curcumin can act on multiple pathways identified in the pathogenesis of AD. It is possible, however, that sporadic AD in humans with the associated cerebral atrophy and neuronal death may be less responsive to curcumin than the AD induced in transgenic animal models of the disease.

As discussed in this review, increasing the bioavailability, BBB penetration and sustaining the half-life of curcumin remains a major focus in relation to its dose–response. To achieve the same degree of efficacy in human studies as compared with animal

studies, closer scrutiny of the administration route and also formulation are required, as increasing bioavailability and BBB penetration is critical. Research analysing the different oral formulations is lacking, and this is an area for further investigation. Furthermore, as the pre-clinical signs of AD are present decades before its clinical onset and most of the late-stage AD clinical trials have recently failed, intervention must be focused at preventing or delaying AD onset. It is reasonable to include healthy community-dwelling older adults and those with subjective memory complaints, in intervention studies with curcumin, for a longer duration with longitudinal follow-up. Last, inclusion of AD-related biomarkers and neuroimaging would add to the clinical significance of curcumin's efficacy in the prevention of AD and associated cognitive decline.

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