Curcumin, a curry spice ingredient, detects and differentiates between pathological tau inclusions in human histological brain sections

Kurkumin, sestavina začimbe curry, označuje vključke patološke beljakovine tau v histoloških rezinah človeških možganov in razločuje med njimi

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Izvleček

Izhodišča: Kurkumin, naravno fluorescentno barvilo in močan protivnetni antioksidant, ki ga kot začimbo in ajurvedsko zdravilo uporabljajo že stoletja, se veže na odlage amiloida beta, alfa-sinukleina in prionov in celo prepreči njihovo agregacijo. V literaturi ni poročil o vezavi kurkumina na tau, protein, ki se patološko spremenjen odlaga pri številnih nevrodegenerativnih boleznih, taupatijah. Namen naše raziskave je bil analizirati kurkuminsko označevanje značilnih patoloških odlag hiperfosforiliranega tau na reprezentativnih primerih treh glavnih skupin taupatij: Alzheimerjeve bolezni (AD; skupina 1), progresivne supranuklearne paralize (PSP; skupina 2) in Pickove bolezni (PiD; skupina 3).

Metode: Ustrezne patološke strukture smo prepoznali v rezinah možganov, pobarvanih s HE, in jih fotografirali. Nato smo analizirali označevanje teh struktur s kurkuminom in AT8 na posnetkih istih prizorov po zaporednem barvanju najprej s kurkuminom in nato z imunofluorescenco na AT8. Rezultate smo izrazili v odstotkih označenih struktur in opisno.

Rezultati: Kurkumin je označil fibrilarne strukture tau pri AD (95 %) in PSP (90 %), ne pa pri PiD. Primerjava barvanja s kurkuminom z imunofluorescenco na AT8 pri AD in PSP je pokazala, da je kurkumin označil fibrilarne AT8-pozitivne strukture, ne pa tistih AT8-pozitivnih sprememb, ki niso kazale fibrilarne zgradbe. Kurkumin je pri AD in PSP označil tudi zunajcelične fibrilarne pentlje, ki ostanejo po odmrtju nevronov, niso se pa prikazale v imunofluorescenci z AT8.

Zaključki: Kurkumin je razločeval med patološkimi odlagami tau na dva načina. Prvič tako, da se je selektivno vezal na odlage tau s fibrilarno zgradbo. In drugič tako, da je razlikoval med fibrilarnimi odlagami tau pri različnih taupatijah: ni označil Pickovih telesc, medtem ko je jasno označil nevrofibrilarne pentlje pri AD in PSP.

Abstract

Background: Curcumin, a natural fluorochrome and a potent anti-inflammatory antioxidant used as a spice and as an ayurvedic remedy for centuries, has been shown to label pathologic aggregates of beta amyloid, alpha-synuclein and scrapie prion protein, and to reduce their aggregation. Curcumin binding to tau, a protein that pathologically aggregates in a wide family of neurodegenerative diseases, tauopathies, has not been examined, yet. Our study was aimed at assessing curcumin labelling of characteristic pathological deposits of hyperphosphorylated tau

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protein in brain sections from representative cases of three major classes of tauopathies: Alzheimer's disease (AD; class 1), progressive supranuclear palsy (PSP; class 2) and Pick's disease (PiD; class 3).

Methods: The structures of interest were first identified in HE-stained sections and photographed. Subsequently, the visualization of these structures with curcumin and AT8 immunofluorescence was assessed by sequential labelling and computer-assisted photography of the same loci. Results were expressed as percentage of structures labelled.

Results: Curcumin detected fibrillar tau in AD (95 %) and PSP (90 %), but not in PiD. When comparing curcumin labelling to AT8 immunofluorescence in AD and PSP, curcumin labelled fibrillar AT8-positive, but not non-fibrillar AT8-immunofluorescent structures. Curcumin also labelled extracellular fibrillar tangles remaining after neuronal death in AD and PSP, which were not visualized by AT8 immunofluorescence.

Conclusions: Curcumin fluorescence was shown to differentiate between pathological tau deposits in two ways. Firstly, it preferentially detected tau deposits with fibrillar morphology. Secondly, curcumin also differentiated between representative cases of main tauopathy classes: it did not reveal fibrillar Pick bodies, while clearly labelling neurofibrillar tangles found in AD and PSP.

Introduction

Curcumin, the active component of spice turmeric, the ground rhizome of Curcuma Longa Lynn, is the molecule that gives curry its yellow colour. Introduced in Europe as a spice in the 14th century,[1] it has been gaining interest of western medicine only recently, although it has been well known as a remedy in eastern cultures for a long time. Over centuries, curcumin has been used in Ayurvedic and traditional Chinese medicine for its antiseptic, analgesic, anti-inflammatory, antioxidant, antimalarial, insect-repellent and wound-healing activities.[1] Modern medicine has confirmed its strong antiinflammatory and antioxidative properties, as well as its action on a wide spectrum of cellular processes that provide the basis for its chemotherapeutic, chemopreventive and chemosensitizing activities.[2] Curcumin effects may be beneficial in cancer therapy and prevention, numerous inflammatory diseases, autoimmune disorders, diabetes mellitus, cardiovascular diseases and neurodegenerative disorders (for review see[1]). Reduced prevalence of Alzheimer disease (AD) in populations on curry-rich diet and positive association between curry consumption and cognitive test performance in elderly Asians (3) were first attributed to the antiinflammatory and antioxidative properties of curcumin. However, it has been shown that curcumin also binds to beta amyloid aggregates in senile plaques of AD *in vivo,* and even reduces their formation and abundance,[4,5] suggesting that it may have a potential to interfere with conformational pathology, which is central to the pathogenesis of numerous neurodegenerative diseases. This possibility was supported by findings that curcumin binds to aggregates of prion protein and inhibits the accumulation of the pathological prion conformer in *vitro.*[6,7] Similar effects were observed also in alpha-synuclein.[8] Curcumin is weakly fluorescent in aqueous solutions – it absorbs light at around 420 nm and emits fluorescence at about 530 nm – but its fluorescence is enhanced with the decrease of the polarity of the medium,[9] and when bound to the protein. The described properties make curcumin a potentially useful tool for detecting conformational pathology of different proteins *in vitro.*

Apart from beta-amyloid, prion protein and alpha-synuclein, there is another protein that undergoes pathologic modifications, accumulates in the brain and is critically involved in neurodegeneration: the microtubule associated protein tau. Hyperphosphorylated tau accumulates in the cytoplasm of neurons and glia, polymerizes and forms fibrillar tau inclusions, of different composition and ultrastructural characteristics typical of different neurodegenerative diseases, called tauopathies (for a recent review, see[10]). Tauopathies often display clinically as dementia, AD being the most prevalent tauopathy.

In the adult human brain, there are six isoforms of tau[11] that fall into two groups, according to the number of microtubule binding domains (MBD) they contain. The ones with three MBD are called 3R-tau and the ones with four MBD are called 4R-tau.[12] Tau fibrillizes into three different types of filaments observed in different fibrillar inclusions: paired helical filaments (PHF), straight filaments (SF) and random coiled filaments (RCF).[13] The isoform and filament type composition varies in different fibrillar inclusions, allowing tauopathies to be divided into 5 classes.[14] The majority of disorders fall into classes 1 to 3. In Class 1, best represented by AD, all six isoforms of tau accumulate and form PHF and SF.[14] The fibrillar tau deposits of Class 2 disorders are composed predominantly of 4R-tau that accumulates in the form of SF.[14] Progressive supranuclear palsy (PSP) is an example of Class 2 tauopathies. The only representative of class 3 disorders is Pick disease (PiD), with predominantly 3R tau accumulating in the form of SF or PHF.[14] Conformational pathology of tau is not only a distinguishing manifestation of tauopathies. Rather, it seems to be at the core of pathogenesis of these numerous neurodegenerative diseases.

Binding of curcumin to tau, suggesting its possible detection of or interference with tau pathology has not been reported, yet. Thus, we performed this pilot investigation to assess curcumin binding to tau deposits on human post mortem brain samples from representative disease cases of the main classes of tauopathies, AD, PSP and PiD, taking advantage of the fluorescent properties of curcumin. To assess curcumin detection of the pathological, aggregated tau in tauopathies, brain sections from representative cases of AD, PSP and PiD were sequentially labelled by hematoxylin-eosin (HE), curcumin and immunofluorescence for hyperphosphorylated tau with AT8 antibody, enabling us to first identify likely pathological structures in HE staining, analyze their labelling with curcumin and eventually verify the presence of specific epitopes of pathologically hyperphosphorylated tau in the same structures.

Materials and Methods

To observe the binding of curcumin to pathological tau in fibrillar inclusions, we used five µm thick deparaffinated sections of paraformaldehyde-fixed, paraffin-embedded human cerebral samples from patients with previously confirmed tauopathies, representative of classes 1, 2 and 3: AD (n = 5), PSP (n = 1) and PiD (n = 1), respectively. They were generously provided from the archive of the Institute of Pathology, Faculty of Medicine, University of Ljubljana by Dr. Mara Popović. In each disease, the typically affected brain region was examined: hippocampus (AD and PiD) and midbrain (PSP). In addition, tissue sections of equally processed brain from a patient with vascular hyalinosis were used as a negative control (i.e., nonfibrillar protein deposition), and brain sections from a patient with cerebral amyloid angiopathy as a positive control for conformational pathology.

The experiment consisted of sequential labelling of the same sections with different techniques allowing direct comparison of these techniques in detection of the same pathological structures, characteristic of tauopathies. Care was taken that the sequential labelling techniques did not interfere with one another and that every signal obtained derived solely from the currently applied label. For this purpose sections were thoroughly destained and the destaining effect was verified by photography of the same loci before the subsequent labelling in the fluorescent filter to be used to photograph the same labelling. All photomicrographs were taken with Nikon DXM2000 camera (Nikon, Tokyo, Japan) attached to Olympus AX-81 fluorescent microscope (Olympus, Tokyo, Japan).

Hematoxylin and eosin (HE) staining

The experiment started with standard HE staining of brain sections. Planar coordinates of the loci were recorded with the images. A minimum of ten (n = 10) loci per section were photographed. HE was then thoroughly destained by incubating the sections in 1 % HCl in 70 % ethanol (EtOH) overnight.

Curcumin labelling

The sections were first permeabilized with 0.3 % Triton X-100 (Sigma-Aldrich Chemie GmbH, Germany) in mili-Q water. Then, curcumin from Curcuma longa (Sigma-Aldrich Chemie GmbH, Germany) was first dissolved in 100 % EtOH to make a 1 mmol/L stock, which was further diluted with mili-Q water to a 10 µmol/L working solution. The sections were incubated in the working solution for 10 minutes, rinsed, differentiated in 70 % EtOH for 1 minute, rinsed and coverslipped with glycerol. Photomicrographs of curcumin labelled sites were taken using filter with excitation/emission wavelengths 420-440/475 nm. Curcumin was destained by incubating the sections in 98 % EtOH overnight.

Immunofluorescence

To detect the presence of pathological hyperphosphorylated tau in the photographed structures, indirect immunofluorescence with the standard monoclonal antibody AT8 (Pierce Endogen, Thermo Fischer Scientific, Rockford, IL, USA;[11]) was performed last. The sections were pretreated by 10-minute pressure cooking in Na-citrate buffer, pH 6.0, and rinsed in buffer, pH 7.2, before a 10 % normal goat serum was applied in buffer, pH 7.2, for 30 minutes to block the nonspecific binding of the secondary antibody. Incubation with the primary antibody (1 : 100) was performed overnight at 4 °C. Finally, the sections were incubated in Alexa 546-conjugated goat anti-mouse secondary antibody (1:750; Molecular Probes, Invitrogen Co.,

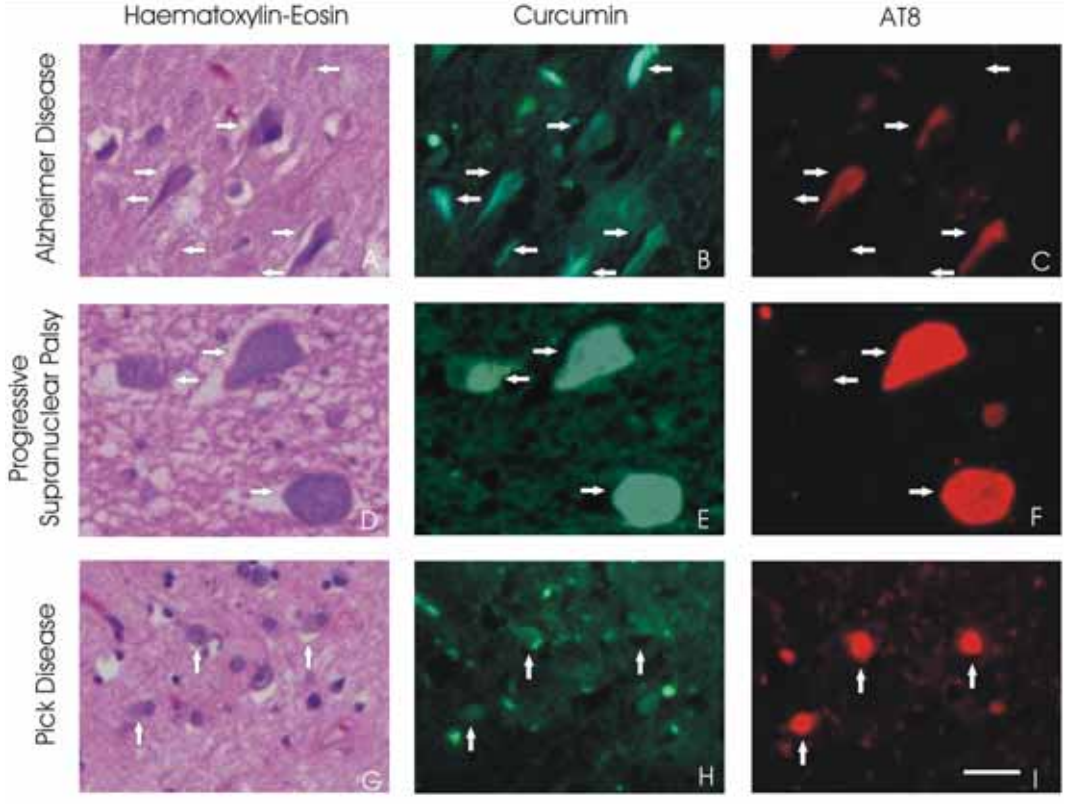

Figure 1: Curcumin labelling of brain sections in Alzheimer's disease (AD; A – C), progressive supranuclear palsy (PSP; B – F) and Pick's disease (PiD; G –I). Curcumin labelled structures in AD (B) that were AT8 (C) positive (right-facing arrows) and fibrillar structures that were AT8 negative (left-facing arrows). Both structures can be observed in HE stained sections: the curcumin and AT8 positive structures (right-facing arrows) are fibrillar and basophilic, while curcumin positive but AT8 negative ones (left-facing arrows) are seen due to their fibrillar structure, but are less basophilic. In PSP, curcumin labelled all of the analyzed structures, labelled with AT8 (E and F, right-facing arrows). However, it also labelled the fibrillar tangle- shaped structures that are AT8 negative (left-facing arrows). Again, both structures are seen in HE staining (D), the double positive (right-facing arrows) are clearly distinguishable, while the curcumin positive and AT8 negative are barely seen in HE (left-facing arrows). There is no curcumin labelling in PiD (G-I). The AT8 positive Pick bodies (I, upwards-facing arrows) are curcumin negative (H, upwards-facing arrows). Bar = 50 µm

Carlsbad, CA, USA). The same loci as in previous labelling runs were photographed using the filter with excitation/emission wavelengths 530-550/590 nm.

Analysis

To assess the binding of curcumin to pathological tau aggregates in representative tauopathies, photomicrographs of the same locus, obtained after each labeling procedure, were analyzed: every structure identified in HE stained section was assessed for its labelling with curcumin and AT8 immunofluorescence. The results were expressed as absolute numbers or percentage of each type of structures labelled with either labelling procedure.

Results

A summary of the labelling outcome with curcumin and AT8 by disease and by structure (as seen in routine HE-stained brain sections) is provided in Table 1. Although the number of analyzed structures in this pilot study was rather low, the results were reproducible.

Curcumin labeled amyloid in positive control samples (amyloid angiopathy), but not the nonfibrillar protein deposits in vascular hyalinosis negative controls.

Curcumin labelled neurofibrillary tangles in AD

In the hippocampal sections of neuropathologically confirmed AD, curcumin clearly labelled intracellular neurofibrillary tangles (NFT; Figure 1 B), previously recognized in HE (Figure 1 A) on the basis of their typical basofilic flame-shape and clear fibrillar morphology. Curcumin labeled 87 % of these structures (n = 27), revealing their fibrillar structure. Curcumin also labeled 100 % (n = 43) of the extracellular structures seen in HE as loosely packed pale eosinophilic fibrils, named "ghost tangles" because they are the only remains of dead neurons.[15]

Presence of hyperphosphorylated tau in the NFT was determined by indirect immunofluorescence with AT8 (Figure 1 C), the antibody routinely used in neuropathological laboratories to assess hyperphosphorylated tau in brain tissue sections, and for AD staging.[16] AT8 labelled intracellular NFT with high intensity and reproducibly. In contrast, AT8 labelled only a small portion (11 %; n = 5) of extracellular ghost tangle population. Indeed these structures were often not visible in AT8 immunofluorescence or displayed only weak and/or punctate immunoreactivity to AT8.

A majority (82 %) of AT8 immunoreactive structures in AD were labelled with curcumin, suggesting that curcumin recognizes most of the pathological tau deposits. The remaining 18 % of AT8 immunoreactive structures not displaying suprathreshold curcumin labelling were seen in HE as neurons with intensely basophilic cytoplasm not showing fibrillar morphology. This AT8 immunoreactivity thus represents nonfibrillized hyperphosphorylated tau accumulating in the neuronal cytoplasm.

On the other hand, curcumin labelling was not predictive of the outcome of the subsequent AT8 IF on the same structures. Only 42 % of curcumin-labelled structures in AD brain tissue sections were also clearly visualized by AT8 IF, while 58 % of curcumin-labelled structures were either weakly or punctately labelled with AT8, or the immunoreactivity was undetectable. The fibrillar nature of these structures was clearly visible in curcumin labelling. These structures appeared fibrillar also in HE (Figure 1 A), and were identified as so-called "ghost" tangles, late stages of evolving fibrillar tau pathology located extracellularly after decay (disintegration) of affected neurons.[15]

Curcumin labelled globose tangles in PSP

Similarly to AD, curcumin readily labelled the intracellular globose tangles as well as the extracellular ghost tangles in PSP (Figure 1 E). Again, the fibrillar structure of tangles was visible in HE and also in curcumin fluorescence. AT8 labelled the intracellular fibrillar tangles extensively while the extracellular tangle labelling appeared less consistent: there was a variability of the AT8 IF signal area and/or intensity compared to

curcumin. Almost all AT8 positive structures were labelled also with curcumin (a rare exception is shown in Figure 1 F). However, similarly to AD, there were 52 % of NFT that were very strongly labelled with curcumin, but only weakly with AT8, or the AT8 immunoreactivity was not detectable (Figure 1 E, F).

Findings from AD and PSP are displayed in a schematic form in Figure 2, which includes a tentative explanation for the divergent trends of curcumin and AT8 labeling in these disorders.

Curcumin did not label Pick bodies in PiD

In PiD, no curcumin labelling of pathological tau Pick bodies was observed (Figure 1 H), although the same Pick bodies (n = 73) were consistently AT8-immunopositive.

Discussion

The present study aimed to assess curcumin labelling of characteristic pathological deposits of hyperphosphorylated tau protein on brain sections from representative cases of three major classes of tauopathies: AD, PSP and PiD.

In AD, the results revealed that curcumin and AT8 labelling overlapped to a high degree, confirming that curcumin labels hyperphosphorylated tau-containing structures. However, there were also significant differences between curcumin and AT8 signals: curcumin reproducibly and intensely labelled both intracellular and extracellular fibrillar structures identified in HE as NFT and ghost tangles, respectively, while the AT8 immunofluorescence labelled the intracellular NTF strongly, but the AT8 signal in the extracellular ghost tangles was predominant-

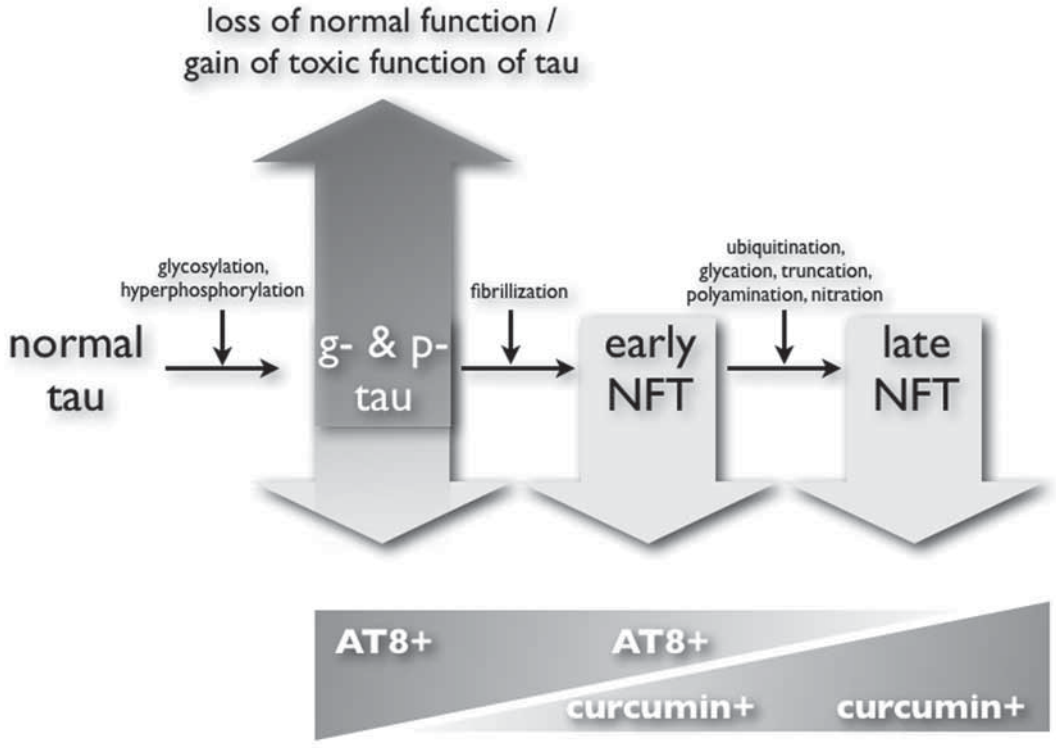

Figure 2: Schematic summary of different post-translational modifications of tau, which contribute to pathology of tauopathies and may explain the results found in Alzheimer's disease and progressive supranuclear palsy in the present study. Abnormal glycosylation (g-tau) and hyperphosphorylation (p-tau) occur early in pathogenesis. P-tau loses its normal activity to stabilize microtubule assembly. On the other hand, it is toxic to the cell. In addition, hyperphosphorylation promotes self-assembly of tau into filaments that generate neurofibrillary tangles (NFT). Fibrillized tau is further modified by ubiquitination, glycation, polyamination, nitration, and proteolytic truncation. Some of these modifications may be key to the loss of labelling with AT8 antibody in step with NFT evolution and concurrent increase in curcumin labelling, as seen in our study.

ly weak, sparse (i.e., punctate in distribution) or undetectable. An additional finding were strongly AT8 immunoreactive neurons, due to nonfibrillar intracellular hyperphosphorylated tau (in HE these cells had intensely basophilic cytoplasm, but no fibrillar inclusions), which was not labeled with curcumin. This finding strongly suggests that curcumin labelling depends on the fibrillar structure of tau deposits and not merely on the presence of hyperphosphorylated tau.

Similar results to AD were obtained in PSP, where the percentage of extracellular ghost tangles labelled with AT8 was even lower than in AD. Curcumin labelled almost all AT8 immunoreactive globose tangles, but more than half of curcumin labelled tangles did not display significant AT8 immunoreactivity.

In Pick bodies, the hallmark lesion of class 3 representative tauopathy – PiD, there was practically no overlap between AT8 immunoreactivity and curcumin fluorescence. Pick bodies were characteristically strongly labelled by AT8, but not detectable by curcumin.

Thus, in this pilot study on curcumin labelling of tau pathology, curcumin fluorescence was shown to differentiate between pathological tau deposits in two ways. First, it preferentially detected tau deposits with fibrillar morphology. Second, curcumin also differentiated between representative cases of main tauopathy classes: it did not reveal fibrillar Pick bodies, while clearly labelling those fibrillar tau structures found in AD and PSP.

To explain how curcumin differentiates between nonfibrillar and fibrillar accumulation of hyperphosphorylated tau in AD and PSP, we would need to know the requirements for curcumin fluorescence upon binding to pathological tau. Our search yielded no published reports on labeling of tau with curcumin, but there may be some analogy with curcumin binding to other misfolded proteins, e.g., beta-amyloid and scrapie prion protein. When tested *in vitro* with A-beta amyloidogenic peptides, curcumin was found to inhibit aggregation of monomeric A-beta and even stimulate disaggregation once it has already occurred, even when curcumin was present at low concentrations.[5] The mechanism of these curcumin effects did not seem to depend on the amyloidogenic peptide sequence but on the fibril-related conformation.[5] These results imply that curcumin could have similar effects on other proteins subject to conformational pathology *in vivo.* When looking at the conformation dependence of curcumin binding to prion protein a recent study found that – in addition to prion fibrils – curcumin bound to a monomeric intermediate and the beta-structured oligomers.[6] Such binding to early pathologic forms was proposed to underlie

Table 1: Curcumin labelling and AT8 immunofluorescence of structures (described by their appearance in hematoxilin-eosin (HE) stained sections) in representative tauopathies. Numbers of all structures of interest found in the analyzed samples and of those that labelled with curcumin and with AT8 immunofluorescence are displayed in respective columns. Curcumin labelled fibrillar structures (both, intra-and extracellular) in AD and PSP, but not in PiD. The extracellular fibrillar structures in AD and PSP, however, were mostly not labelled with AT8. Curcumin also did not label the nonfibrilar intracellular accumulations of hyperphosphorylated tau in AD and PSP.

tauopathy	structures in HE	curcumin	AT8
Alzheimer disease (AD)	Intracellular nonfibrillar n = 10	0	10
	Intracellular fibrillar n = 31	27	31
	Extracellular fibrillar n = 40	40	4
Progressive supranuclear palsy (PSP)	Intracellular nonfibrillar n = 1	0	1
	Intracellular fibrillar n = 21	19	19
	Extracellular fibrillar n = 17	17	7
Pick disease (PiD)	Intracellular fibrillar n = 73	0	73

curcumin's demonstrated ability to inhibit the conversion of the native protein to pathological prion.[7] There are indications that tau may fibrillize via an oligomeric intermediate[17,18] allowing for a testable hypothesis that curcumin might be able to bind to these early stages and inhibit polymerization of tau.

Our findings suggest that: (1) there is a progressive disappearance of the AT8 tau epitope through tangle maturation and transition from the early (intracytoplasmic) to the late (extracellular, ghost) NFT, and (2) the same post-translational processing of tau that contributes to its fibrillization facilitates curcumin binding and fluorescence, enabling better detection of later stages of tangle evolution, ghost tangles, with curcumin than with AT8 (Figure 2). Our results are in agreement with findings of Uchihara and coworkers, who have employed a different fluorophore, thiazin red, and compared it to silver impregnation and AT8 immunodetection of NFT. Their results also reveal AT8 failure to detect late stages of NTF evolution.[19] Interestingly, this failure of AT8 to visualize a significant proportion of pathological tau deposits had been shown by other groups in the past[20] but it has not hampered the use of AT8 as the golden standard diagnostic tool for detecting tau pathology. Indeed, current staging of AD is based on AT8 immunodetection of tau pathology in brain sections.[16]

As pointed out in the introduction, classes of tauopathies differ both in the isomer composition of tau deposits and in the ultrastructure of the accumulating fibrils. Our case of class 3 tauopathy, Pick disease, displayed highly reproducible absence of fibrillary Pick body labelling with curcumin. Based on the methods employed in this study – we cannot determine whether this was due to the biochemical or structural characteristics of Pick bodies. Although curcumin binding to A-beta amyloidogenic peptides was shown not to depend on their primary sequence,[5] it is plausible that 3R and 4R tau isomers differ in their fibrillogenic potential and in the posttranslational modifications of the fibrils, which could lead to differential labelling with curcumin. Support for this tentative explanation is provided by evidence that 4R tau was found to be easier to phosphorylate and aggregate into filaments than 3R tau,[21] and that both standard Gallyas silver impregnation and amyloid-labelling fluorophore thiazin red fail to label Pick bodies.[22] It is possible that 3R-tau composed fibrils undergo a different extent or pattern of posttranslational modification, obscuring or removing the binding sites for Gallyas silver impregnation, thiazin red and curcumin.

Our study provided an encouraging first evidence of curcumin binding to tau deposits in the human brain. However, in view of curcumin potential to detect and influence progression of conformational pathology *in vitro* and *in vivo,* a more detailed analysis is needed both on post mortem brain sections from these and other tauopathies, at the biochemical level on recombinant tau, and in experimental animals expressing tau pathology. A larger number of tauopathy cases should be analyzed to reassess and potentially validate our findings. Furthermore, curcumin labelling needs to be better characterized by careful comparison to established fluorochromes (e.g. thioflavin S or thiazin red) and other labelling procedures, routinely used for detection of fibrillary pathology (e.g. Gallyas silver impregnation). To address the question whether curcumin labelling correlates with the presence of tau isomeres in the deposits, a colocalization analysis of curcumin fluorescence with 3R and 4R tau immunolabelling should be performed. The possibility that the failure of curcumin to label Pick bodies is due to some ultrastructural characteristics of these fibrillar tau inclusions, should also be addressed. In addition, *in vitro* binding assays should look at curcumin-tau interactions at various stages of conformational transformation and fibrillization to reveal structural requirements for curcumin labelling and to determine whether curcumin can interfere with aggregation of pathological tau. Finally, curcumin-rich diet should be tested in animal models of tauopathies for its ability to stave off or prevent development of conformational pathology of tau. Ideally, however, curcumin should be able to counteract the toxic effects of hypephosphorylated tau oligomers and to prevent neurodegeneration.

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