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Curcumin improves tau-induced neuronal dysfunction of nematodes

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ABSTRACT

Tau is a key protein in the pathogenesis of various neurodegenerative diseases, which are categorized as tauopathies. Because the extent of tau pathologies is closely linked to that of neuronal loss and the clinical symptoms in Alzheimer's disease, anti-tau therapeutics, if any, could be beneficial to a broad spectrum of tauopathies. To learn more about tauopathy, we developed a novel transgenic nematode (*Caenorhabditis elegans*) model that expresses either wild-type or R406W tau in all the neurons. The wild-type tau-expressing worms exhibited uncoordinated movement (Unc) and neuritic abnormalities. Tau accumulated in abnormal neurites that lost microtubules. Similar abnormalities were found in the worms that expressed low levels of R406W-tau but were not in those expressing comparative levels of wild-type tau. Biochemical studies revealed that tau is aberrantly phosphorylated but forms no detergent-insoluble aggregates. Drug screening performed in these worms identified curcumin, a major phytochemical compound in turmeric, as a compound that reduces not only Unc but also the neuritic abnormalities in both wild-type and R406W tau-expressing worms. Our observations suggest that microtubule stabilization mediates the antitoxicity effect of curcumin. Curcumin is also effective in the worms expressing tau fragment, although it does not prevent the formation of tau-fragment dimers. These data indicate that curcumin improves the tau-induced neuronal dysfunction that is independent of insoluble aggregates of tau.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia among the elderly (Mayeux and Stern, 2012). Because of the increasing prevalence of AD with age and rapid extension of life-span, it is urgent to develop prevention and therapeutic strategies against AD (Mangialasche et al., 2010). A characteristic pathological change of AD is the formation of tau inclusions, which consist of neurofibrillary tangles and neuropil threads, in the areas of the brain where neuronal loss occurs (Braak and Braak, 1997; Gomez-Isla et al., 1997). It is believed that long-term Aβ deposition triggers the death cascade of tau, which eventually leads to neuronal death (Goate and Hardy, 2012). Several published reports using animal models have supported this amyloid cascade hypothesis (Gotz et al., 2001; Lewis et al., 2001).

Various anti-amyloid strategies have been developed, including Aβ immunization, anti–Aβ-aggregation compounds, β- or γ-secretase inhibitors, and γ-secretase modulators (Mangialasche et al., 2010). Although some of these strategies were shown to be effective in clearing the pre-existing Aβ-aggregates and preventing further Aβ deposition and its associated toxicity, clinical studies have reported that these strategies had at most limited efficacy on cognitive dysfunction of symptomatic patients (Corbett et al., 2012; Holmes et al., 2008; Selkoe, 2012). In contrast, anti-tau therapy, if any, could lead to clinical improvement in patients, as tau pathologies are closely linked to the levels of cognition in AD (Delacourte et al., 1999; Gomez-Isla et al., 1997). In addition, as the tau pathologies are associated with a number of age-related neurodegenerative diseases other than AD (Lee et al., 2001), anti-tau therapy would have beneficial effects on a broad spectrum of dementias.

The mechanisms of tau-associated neurodegeneration still remain unclear. Thus, current anti-tau strategies mostly target the aggregation of tau, which is believed to be toxic to neurons (Boutajangout et al., 2011). In fact, it is claimed that methylene blue,

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a potent tau-aggregation inhibitor, prevented the progression of clinical symptoms (Wischik and Staff, 2009). However, it is not yet known if aggregation or inclusion formation of tau is responsible for the pathogenesis of tauopathies.

One way to efficiently screen for drugs is to test naturally occurring compounds that have been validated epidemiologically. Curcumin, a yellow phytochemical in the rhizome of *Curcuma longa*, which has various biological effects (Belkacemi et al., 2011), has been used as a spice in India. Epidemiological studies reported that the prevalence of AD in India is less than that in the US, which may suggest that a curcumin-rich diet might decrease the risk of developing AD (Chandra et al., 2001). We thus hypothesized that curcumin may attenuate tau-induced neurodegeneration.

In this study, we developed several *Caenorhabditis elegans* models of tauopathy in which the neurons express human tau. We observed tau-dependent neuronal dysfunction and morphological abnormalities even in the absence of insoluble tau-aggregates. Furthermore, curcumin is effective in treating the tau-induced neuronal dysfunction.

2. Materials and methods

2.1. Development and maintenance of worm strains

Human tau, tau fragment, or DsRed complementary DNA (cDNA) was subcloned downstream of the "pan-neuronal" unc-119 promoter (Punc-119) sequence in the pFXneo-punc119 vector. For the Punc-119-driven DsRed transgenic (Tg) worms, the expression vector was diluted with the pBluescript vector and injected into wild-type worm, N2. For the Punc-119-driven tau-Tg worms, the vectors were coinjected into N2 with the marker pFX-Pges-1:enhanced green fluorescent protein. Germline transformation and generation of genome-integrated Tg lines were performed using ultraviolet irradiation, as described previously (Mello and Fire, 1995; Mello et al., 1991; Mitani, 1995). The dose of radiation for DNA integration was 250 J/m^2 or 300 J/m^2. The integrated Tg lines were backcrossed to N2 5 (for tau) or 2 (for DsRed) times before analysis. The Tg nematodes contained multiple copies of the transgene. The Tg strains used are as follows; Mock-Tg (tmIs388), WT4R-Tg (tmIs389 and tmIs390), WT3R-Tg (tmIs252), P301L-Tg (tmIs227), R406W-Tg (tmIs226), 4R-tau C-terminal fragment-Tg (CT4R, tmIs712), and DsRed-Tg (tmIs591) (Xie et al., 2014). For morphological studies, the following tau-Tg lines that were crossbred with the DsRed-Tg line were used: Mock/DsRed-Tg (tmIs388/591), WT4R/DsRed-Tg (tmIs390/591), and R406W/DsRed-Tg (tmIs226/591). All the *C. elegans* strains were maintained on the nematode growth medium (NGM)-containing plates that were spread with *Escherichia coli* OP50 under standard conditions according to Brenner with modifications by Way and Chalfie (Brenner, 1974; Way and Chalfie, 1988).

2.2. Behavioral analyses

Healthy adult worms were transferred to a new NGM plate to lay eggs. Parent worms were picked off the plate 2 hours later, and the resulting synchronized eggs were cultivated at 20 °C until isolation. For isolation, worms at varying stages were selected individually and placed on new 3.5-cm plates. For the estimation of uncoordinated movement (Unc), worms were observed under a microscope. The severity of Unc was scored as follows: normal, wide bending with fast movement; Unc, narrow bending with slow movement; severe Unc, narrow bending with slower or negligible movement. Liquid thrashing assays were performed as previously described with minor modifications (Kraemer et al., 2003). The worms were placed in a drop of M9 buffer (22-mM KH_2PO_4, 42-mM Na_2HPO_4, 85-mM NaCl, 1-mM $MgSO_4$) on a glass slide, and the thrashing movements were counted for 30 seconds under a microscope. All the assays were performed blindly. Twenty worms were used in each assay for each experiment, and the experiment was performed independently 3 to 4 times. Thus, 60 to 80 worms were assessed for each line. The touch assay was performed as described previously (Miyasaka et al., 2005a). The number of responses to 10 touch trials, 5 for the anterior and 5 for the posterior, were counted.

2.3. Biochemical analyses

Synchronized worms were harvested in M9 buffer and pelleted by brief centrifugation. After washing with M9 buffer, the worm pellets were weighed and stored at −80 °C. For the preparation of the total worm lysates, the pellets were sonicated in the sodium dodecyl sulfate (SDS) sample buffer (80-mM Tris-HCl, 2% SDS, 10% glycerol, 1% 2-mercaptoethanol, pH 6.8) and cleared by ultracentrifugation at 150,000× *g* for 10 minutes at 20 °C. The SDS-soluble fractions were used for Western blot analysis as described previously (Miyasaka et al., 2005b). The protein concentration for each sample was normalized by Coomassie Brilliant Blue (Wako Pure Chemical Industries, Ltd, Osaka, Japan) staining on SDS-polyacrylamide gel electrophoresis gels. The detergent solubility of tau was analyzed by sequential solubilization as described previously with minor modifications (Kraemer et al., 2003). Briefly, worm pellets were homogenized using a glass-teflon homogenizer in high salt re-assembly buffer (RAB) (0.1-M 2-Morpholinoethanesulfonic acid, 0.5-mM MgSO4, 1-mM EGTA, 0.5-M NaCl, pH 6.8) supplemented with protease and phosphatase inhibitors followed by centrifugation at 3000× *g* for 3 minutes to remove the unhomogenized worms (Hasegawa et al., 1992; Miyasaka et al., 2005b). The resulting supernatants were further centrifuged at 150,000× *g* for 20 minutes, and the pellets were homogenized in radioimmunoprecipitation assay (RIPA) buffer (50-mM Tris, 150-mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, pH 8.0) and centrifuged at 150,000× *g* for 20 minutes. The residual RIPA-insoluble pellets were sonicated in the SDS sample buffer (RIPA-insoluble fraction). The aliquots from the RAB- and RIPA-soluble supernatants were mixed with the SDS sample buffer and used for Western blot analysis. The microtubules and free tubulin were prepared as described previously (Miyasaka et al., 2010). Briefly, freshly harvested worms were homogenized in a buffer containing taxol and Guanosine triphosphate and subjected to ultracentrifugation. The supernatants and pellets, which consisted of free tubulin and microtubule fractions, respectively, were analyzed by Western blotting. For the absolute quantification, worm tubulin and recombinant human tau (4-repeat 383-amino acid isoform) were purified as described previously (Aamodt and Culotti, 1986; Aoyagi et al., 2007).

2.4. Morphological analyses

For the morphological study, the tau/DsRed-Tg worms were placed into a drop of M9 buffer containing 50-mM NaN_3 on agar pads and covered with a cover glass. For immunocytochemical analysis, synchronized worms at various ages were harvested and kept in 4% paraformaldehyde at 4 °C for 24 hours. The fixed worms were dehydrated with 100% ethanol, immersed in 100% xylene, and embedded in a paraffin block. Paraffin-embedded sections at a 6-μm thickness were processed using a microtome and placed onto MAS-coated glass slides (Matsunami Glass Ind, Ltd, Osaka, Japan). Deparaffinized and hydrated sections were dipped into the target retrieval solution (DAKO, Glostrup, Denmark) at 95 °C for 15 minutes and then blocked with 5% bovine serum albumin in tris-buffered saline (TS; 50-mM tris-HCl, 150-mM NaCl, pH 7.6). The