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Featured Article

Extracellular low-n oligomers of tau cause selective synaptotoxicity without affecting cell viability

Senthilvelrajan Kaniyappan[a,b], Ram Reddy Chandupatla[a,b], Eva-Maria Mandelkow[a,b,c], Eckhard Mandelkow[a,b,c,*]

[a]*DZNE, German Center for Neurodegenerative Diseases, Bonn, Germany*
[b]*MPI for Metabolism Research, Hamburg, Germany*
[c]*CAESAR Research Center, Bonn, Germany*

Abstract

Introduction: Tau-mediated toxicity in Alzheimer's disease is thought to operate through low-n oligomers, rather than filamentous aggregates. However, the nature of oligomers and pathways of toxicity are poorly understood. Therefore, we investigated structural and functional aspects of highly purified oligomers of a pro-aggregant tau species.

Methods: Purified oligomers of the tau repeat domain were characterized by biophysical and structural methods. Functional aspects were investigated by cellular assays ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) bromide assay of cell viability, lactate dehydrogenase release assay [for cell toxicity], reactive oxygen species production, and calcium assay), combined with analysis of neuronal dendritic spines exposed to oligomers.

Results: Purified low-n oligomers are roughly globular, with sizes around 1.6 to 5.4 nm, exhibit an altered conformation, but do not have substantial β-structure. Treatment of primary neurons with oligomers impairs spine morphology and density, accompanied by increased reactive oxygen species and intracellular calcium, but without affecting cell viability (by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) bromide assay of cell viability and lactate dehydrogenase release assay [for cell toxicity]).

Discussion: Tau oligomers are toxic to synapses but not lethal to cells.

Keywords: Tau; Oligomers; Structure; Toxicity; Neurons; ROS production; Calcium elevation; Synapses; Transgenic mouse brain-derived oligomers

1. Introduction

Tau is a natively unfolded protein in solution and forms aggregates in several neurodegenerative diseases collectively called tauopathies, including Alzheimer's disease (AD). AD is characterized by extracellular plaques comprising amyloid β (Aβ) and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau [1]. The nature of Aβ-induced toxicity and its relationship to tau has been explored in several animal models of AD [2,3]. The results suggested that Aβ-induced toxicity is largely dependent on tau. In the absence of tau, the toxic effects of Aβiare ameliorated [4,5]. However, the pathway(s) by which tau causes synaptic and neuronal decay is still poorly understood.

Under pathological conditions, short motifs in the repeat domain of tau adopt a β-sheet conformation, which induces self-assembly with other tau molecules, leading to the formation of insoluble aggregates [6]. It is well established that there is a direct correlation between the distribution of

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*Corresponding author. Tel.: +49-228-43302-610; Fax: +49-228-42202-699.

E-mail address: Mandelkow@dzne.de

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NFTs in the brain and the progression of AD (Braak stages [7]), but in AD patients, the degree of neuronal loss exceeds strongly the accumulation of NFTs. Moreover, synapse loss and microgliosis also precede tangle formation [8,9]. The discrepancy can be reconciled by the fact that memory loss and cognitive deficits correlate with soluble oligomeric tau [10,11]. Consistent with this, mouse models expressing mutated tau such as P301L, P301S, and ΔK280 showed memory loss and neuronal loss even before the robust formation of NFTs [12–14]. These findings suggest that NFTs may not be the primary toxic form of tau aggregates and that intermediate oligomeric species could be responsible for the disruption of normal functions of neurons [15].

Tau oligomers can be heterogeneous; they range from tau dimers to prefibrillar and nonfibrillar forms. The terminology for tau oligomers is not well defined; they may be "on-pathway" (compatible with further fibrillar elongation) or "off-pathway" (not compatible with fibrous growth). They are usually defined in terms of their solubility in detergents or aqueous solution. Depending on conditions, soluble oligomers may contain several dozen protein molecules, including short fibrils. Tau oligomers are in a dynamic equilibrium with their subunits, which makes their preparation difficult in vitro. The hexapeptide motifs in the second (PHF6*) and third (PHF6) repeat regions of tau are responsible for the aggregation of tau into β-sheet structured fibers [6]. During filament assembly, the levels of cysteine-dependent tau oligomers increase, which indicates that intermolecular disulfide cross-linking along with PHF6 hexapeptide motifs may facilitate tau oligomerization [16]. However, anti-aggregant disulfide cross-links can occur as well [17]. Certain types of tau oligomers can be generated in vitro by cross-seeding with other amyloidogenic proteins like Aβ and α-synuclein [18] or by chemical cross-linking [19]. Tau oligomers prepared from full-length human tau have been reported to cause neuronal death in the human neuroblastoma SH-SY5Y cell line when applied extracellularly [20]. Injection of in vitro–prepared tau oligomers into mouse brains can lead to anterograde memory deficits and caspase-mediated cell death [21]. AD brain–derived tau oligomers injected into mouse brains can induce the spreading of tau between neurons [22]. Taken together, these data suggest that tau oligomers are responsible for some aspects of tau-dependent pathology. However, the preparation of oligomers and the criteria for the toxicity measurements were heterogeneous. Therefore, detailed analysis of toxic effects of tau oligomers is necessary.

We have previously generated regulatable mouse lines expressing pro-aggregant and anti-aggregant forms of the tau repeat domain ($Tau^{RD\Delta K}$ or $Tau^{RD\Delta K-PP}$, which either enhances the β-propensity or delete it, respectively) to demonstrate that tau pathology is closely linked to its potential for aggregation. $Tau^{RD\Delta K}$ aggregates rapidly and exhibits pronounced toxicity in cell culture, slice culture, *Caenorhabditis elegans*, and mouse models [23–26]. The toxic effects of $Tau^{RD\Delta K}$ are reversible when the expression of mutant tau is switched off [23,25]. However, it has been unclear whether oligomers of $Tau^{RD\Delta K}$ protein cause the toxicity observed in these models. In vitro studies suggested that $Tau^{RD\Delta K}$ can aggregate without the requirement of polyanionic additives such as heparin [27], arguing that the assembly forms might mimic the natural aggregates in AD and other tauopathies. In the present study, to characterize the toxic species of tau in more detail, we prepared well-defined species of $Tau^{RD\Delta K}$ oligomers without heparin and investigated their role in vitro and in cell models.

2. Materials and methods

2.1. Chemicals

Chemicals of highest quality were purchased from the following suppliers: Sigma, Merck, GERBU, Amersham Pharmacia Biotech, AppliChem, Molecular Probes, Fluka, and Serva.

2.2. Antibodies

The antibodies used were tau antibody K9JA (A-0024; Dako), MAP2 antibody (ab5392-25; Abcam), PSD-95 antibody (MA1-046; Thermo Fischer Scientific), GluR1 antibody (AB-1504; Millipore), drebrin antibody (ADI-NBA-110-E; Enzo Life Sciences), and NeuN (marker of Q3 neuronal nuclei, MAB 377; Millipore). A novel rat monoclonal oligomer–specific antibody 6H1 was generated in our laboratory (to be described elsewhere).

2.3. Preparation of recombinant protein

$Tau^{RD\Delta K}$ in the pNG2 vector was expressed in *Escherichia coli* BL21 (DE3) cells, and protein was prepared as described by Barghorn et al. [28], making use of the heat stability and FPLC SP Sepharose chromatography. In brief, the Q4 cell pellet was resuspended in the extraction buffer (20 mM MES, 500 mM NaCl, 1 mM $MgSO_4$, 1 mM EGTA, 5 mM dithiothreitol (DTT), and 0.2 mM PMSF, pH 6.8 supple- Q5 mented with protease inhibitor cocktail). The cells were disrupted with a French pressure cell followed by heating at 96°C for 20 minutes and centrifugation. The supernatant was dialyzed against two changes of Mono S A buffer (20 mM MES, 50 mM NaCl, 1 mM $MgSO_4$, 1 mM EGTA, 2 mM DTT, and 0.1 mM PMSF, pH 6.8) and centrifuged again. The supernatant was loaded on an ÄKTA Explorer 100 FPLC unit fitted with an SP Sepharose HP column. The protein was eluted with a linear gradient of Mono S B buffer (20 mM MES, 1 M NaCl, 1 mM $MgSO_4$, 1 mM EGTA, 2 mM DTT, and 0.1 mM PMSF, pH 6.8). The purity of the protein was analyzed by SDS-PAGE. The protein was Q6 concentrated using a centrifugal filter with a 3-kDa molecular weight cutoff, and the concentrations were determined by the BCA method. The samples were frozen in liquid nitrogen Q7 and stored at −80°C until further use.