Tauroursodeoxycholic acid suppresses amyloid β-induced synaptic toxicity *in vitro* and in APP/PS1 mice

Rita M. Ramalho[a], Ana F. Nunes[a], Raquel B. Dias[b,c], Joana D. Amaral[a,d], Adrian C. Lo[e],
Rudi D'Hooge[e], Ana M. Sebastião[b,c], Cecilia M.P. Rodrigues[a,d,*]

[a] *Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal*
[b] *Institute of Pharmacology and Neurosciences, Faculty of Medicine, University of Lisbon, Lisbon, Portugal*
[c] *Unit of Neurosciences, Instituto de Medicina Molecular, University of Lisbon, Lisbon, Portugal*
[d] *Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal*
[e] *Laboratory of Biological Psychology, University of Leuven, Leuven, Belgium*

Abstract

Synapses are considered the earliest site of Alzheimer's disease (AD) pathology, where synapse density is reduced, and synaptic loss is highly correlated with cognitive impairment. Tauroursodeoxycholic acid (TUDCA) has been shown to be neuroprotective in several models of AD, including neuronal exposure to amyloid β (Aβ) and amyloid precursor protein (APP)/presenilin 1 (PS1) double-transgenic mice. Here, we show that TUDCA modulates synaptic deficits induced by Aβ *in vitro*. Specifically, TUDCA reduced the downregulation of the postsynaptic marker postsynaptic density-95 (PSD-95) and the decrease in spontaneous miniature excitatory postsynaptic currents (mEPSCs) frequency, while increasing the number of dendritic spines. This contributed to the induction of more robust and synaptically efficient neurons, reflected in inhibition of neuronal death. *In vivo*, TUDCA treatment of APP/PS1 mice abrogated the decrease in PSD-95 reactivity in the hippocampus. Taken together, these results expand the neuroprotective role of TUDCA to a synaptic level, further supporting the use of this molecule as a potential therapeutic strategy for the prevention and treatment of AD.
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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by cognitive decline and loss of memory function (Goedert and Spillantini, 2006). More than 35 million people worldwide are afflicted with AD, and these numbers are expected to quadruple by 2050 (Hebert et al., 2003). Amyloid β (Aβ)-containing plaques, hyperphosphorylated tau-containing neurofibrillary tangles, reduced synaptic density, and neuronal loss in selected brain areas are key histopathological features of AD (Götz et al., 2004).

Although evidence suggests that the accumulation of Aβ plays a central and initial role in AD, synapses are considered the earliest site of pathology. In fact, cortical and hippocampal synapse density is reduced early in the disease process, and synaptic loss is the best pathological correlate of cognitive impairment in AD (Gouras et al., 2010; Terry et al., 1991). Several studies with synthetic Aβ oligomers or natural soluble oligomeric Aβ, purified from the culture media of cells expressing mutant human amyloid precursor protein (APP), or extracted directly from the brains of AD patients, have shown potent synaptic damaging effects (Shankar et al., 2008; Walsh et al., 2000, 2002). In addition, transgenic mice overexpressing wild-type APP or mutated APP associated with increased Aβ showed alterations in

synaptic transmission and plasticity that preceded neuronal death and plaque formation (Chapman et al., 1999; Freir et al., 2001). Importantly, Aβ oligomers can reduce long-term potentiation (LTP), a form of synaptic plasticity that is closely related with learning and memory, and specifically affected in AD (Selkoe, 2002; Shankar et al., 2008). Oligomeric Aβ has also been shown to facilitate the induction of long-term depression (LTD) in hippocampal synapses (Shankar et al., 2008). Impairments in LTP and facilitation of LTD culminate in synaptic depression and impairment in neuronal networks (Palop and Mucke, 2010). The effects of Aβ on synaptic physiology are correlated with structural changes in synaptic morphology. In fact, oligomeric Aβ-mediated inhibition of LTP and enhancement of LTD lead to dendritic spine loss as a result of F-actin remodeling (Selkoe, 2008).

Ursodeoxycholic acid and its taurine-conjugated derivative, tauroursodeoxycholic acid (TUDCA), are endogenous bile acids that increase the apoptotic threshold in several cell types (Rodrigues et al., 1998, 2000). We have demonstrated that TUDCA is neuroprotective in animal models of Huntington's disease (Keene et al., 2001, 2002) and in ischemic and hemorrhagic stroke (Rodrigues et al., 2002, 2003). TUDCA also improved survival and function of nigral transplants in the rat (Duan et al., 2002) and reduced mitochondrial dysfunction in *C. elegans* models of Parkinson's disease (Ved et al., 2005). Importantly, we have previously shown that TUDCA is capable of preventing Aβ-induced apoptosis in different models of AD, including primary cortical neurons and cell lines (Ramalho et al., 2004, 2006; Solá et al., 2003; Viana et al., 2009). By interfering with caspase-3 activation, TUDCA also prevents toxic downstream cleavage of tau (Ramalho et al., 2008). Moreover, we have recently demonstrated that TUDCA is capable of reducing Aβ deposits, glial activation, and neuronal integrity loss in the APP/presenilin 1 (PS1) double-transgenic mouse model of AD, rescuing memory and learning deficits (Nunes et al., 2012).

Using primary rat cortical and hippocampal cultures exposed to Aβ, and brain tissue of APP/PS1 transgenic mice, we sought to explore the protective role of TUDCA at the synaptic level. Aβ_{1-42} is the major component of amyloid plaques in AD brains, whereas Aβ_{25-35} corresponds to an 11 amino acid fragment of Aβ_{1-40} and Aβ_{1-42}. Aβ_{25-35} consists of an intermembrane domain of APP, hampering its production through typical processing (Kang et al., 1987). Nevertheless, Aβ_{25-35} represents the biologically active region of Aβ and has been widely recognized as a model of full-length Aβ. In fact, pure Aβ_{25-35} peptide aggregates with time, forming fibrils with β-structure (Del Mar Martínez-Senac et al., 1999), and retains the toxicity of full-length peptide (Pike et al., 1995). In addition, Aβ_{25-35} induces the same molecular and cellular dysfunction as Aβ_{1-42}, similar to that observed in AD brains [reviewed in (Kaminsky et al., 2010)]. Importantly, it has been shown

that Aβ_{25-35} accumulates in a racemized form (L- to D-Ser[26]), a typical age-dependent modification in AD, suggesting that it can be produced in brains when the soluble racemized Aβ_{1-42} is proteolytically cleaved (Kubo et al., 2002).

Our results showed that TUDCA suppresses Aβ-induced decrease of the neuronal marker postsynaptic density-95 protein (PSD-95). Moreover, TUDCA prevented the reduction in dendritic spine number and the decrease in the frequency of spontaneous excitatory synaptic activity. These results further expanded the neuroprotective role of TUDCA, highlighting its use as a potential therapeutic strategy for the prevention and treatment of AD.

2. Methods

2.1. Isolation and culture of rat neurons

Primary cultures of rat cortical and hippocampal neurons were prepared from 17- to 18-day-old fetuses of Wistar rats, as previously described (Brewer et al., 1993) with minor modifications. In short, pregnant rats were CO_2-anesthetized and decapitated. The fetuses were collected in Hank's balanced salt solution (HBSS-1; Invitrogen, Grand Island, NY, USA) and rapidly decapitated. After removal of meninges and white matter, the brain cortex and hippocampus were collected in Hank's balanced salt solution without Ca^{2+} and Mg^{2+} (HBSS-2). The cortex and hippocampus were then mechanically fragmented, transferred to a 0.025% trypsin in HBSS-2 solution, and incubated for 15 minutes at 37 °C. After trypsinization, cells were washed twice in HBSS-2 containing 10% fetal calf serum (FBS) and resuspended in Neurobasal medium (Invitrogen), supplemented with 0.5 mM L-glutamine, 25 μM L-glutamic acid, 2% B-27 supplement (Invitrogen), and 12 mg/mL gentamicin. Neurons were then plated on tissue culture plates, precoated with poly-D-lysine (Invitrogen) at 1×10^6 cells/mL, and maintained at 37 °C in a humidified atmosphere of 5% CO_2. Half of culture medium was replaced every 3–4 days. All experiments were performed on cells cultured for 2–3 weeks. Isolated rat neurons were incubated with active fragment 25 μM Aβ_{25-35} (Bachem AG, Budendorf, Switzerland) for 2–24 hours, with or without 100 μM TUDCA (Sigma-Aldrich, St. Louis, MO, USA), or no addition. In coincubation experiments, TUDCA was added to neurons 12 hours prior to incubation with Aβ_{25-35}. The predominant aggregates in Aβ preparations are thought to be low N-oligomers, mainly monomeric to tetrameric, as fibrillogenesis usually requires longer incubation times and higher concentrations of the peptides (Stine et al., 2003). In selected experiments, isolated rat neurons were incubated with 2 μM Aβ_{1-42} (Bachem AG), in the presence and absence of TUDCA. Adequate controls using Aβ_{35-25} and Aβ_{42-1} reverse peptides (Bachem AG) were also performed.

Cells were characterized by phase-contrast microscopy and indirect immunocytochemistry for microtubule-associ-