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Tauroursodeoxycholic acid (TUDCA) supplementation prevents cognitive impairment and amyloid deposition in APP/PS1 mice

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease hallmarked by extracellular $A\beta_{1-42}$ containing plaques, and intracellular neurofibrillary tangles (NFT) containing hyperphosphorylated tau protein. Progressively, memory deficits and cognitive disabilities start to occur as these hallmarks affect hippocampus and frontal cortex, regions highly involved in memory. Connective tissue growth factor (CTGF) expression, which is high in the vicinity of Aβ plaques and NFTs, was found to influence γ-secretase activity, the molecular crux in $A\beta_{1-42}$ production. Tauroursodeoxycholic acid (TUDCA) is an endogenous bile acid that downregulates CTGF expression in hepatocytes and has been shown to possess therapeutic efficacy in neurodegenerative models. To investigate the possible *in vivo* therapeutic effects of TUDCA, we provided 0.4% TUDCA-supplemented food to APP/PS1 mice, a well-established AD mouse model. Six months of TUDCA supplementation prevented the spatial, recognition and contextual memory defects observed in APP/PS1 mice at 8 months of age. Furthermore, TUDCA-supplemented APP/PS1 mice displayed reduced hippocampal and prefrontal amyloid deposition. These effects of TUDCA supplementation suggest a novel mechanistic route for Alzheimer therapeutics.

Introduction

Brain deposition of amyloid-beta (Aβ) is a central pathobiochemical event in Alzheimer's disease (AD). The Aβ cascade hypothesis explains how cleavage of amyloid precursor protein (APP) by the infamous γ-secretase complex (Li et al., 2009) produces toxic soluble Aβ monomers and oligomers that aggregate into amyloid deposits, and could gradually lead to widespread neural and glial dysfunction, memory defects, and ultimate dementia (Hardy and Selkoe, 2002; Selkoe, 2008; Walsh and Selkoe, 2004). Braak and Braak (1991) historically showed that amyloid deposits first occur in basal portions of the frontal, temporal and occipital isocortex of the AD brain. Distinct phases of Aβ deposition have been identified starting in isocortex, soon spreading to hippocampus and other allocortical regions, and eventually involving vast areas of the brain (Thal et al., 2002). By and large, Aβ neuropathology first seems to hit brain regions that are important for cognition (including learning and memory), and affect regions that play a role in other brain functions in later stages of the disease (Bero et al., 2011; Jucker and Walker, 2011; Pievani et al., 2011).

Ursodeoxycholic acid (UDCA) and its taurine conjugate (TUDCA) are endogenous bile acids that are able to cross the blood–brain barrier and exert their effects on the central nervous system (Keene et al., 2001; Parry et al., 2010). Parry and colleagues reported dose-dependent increases in UDCA serum and cerebrospinal fluid concentrations in amyotrophic lateral sclerosis (ALS) patients after UDCA administration (Parry et al., 2010). Also TUDCA enters the brain after systemic administration as TUDCA brain levels increased up to 6-fold in TUDCA-treated rats (Keene et al., 2001). Furthermore, TUDCA displayed neuroprotective activity in cellular AD models (Ramalho et al., 2008) as well as *in vivo* models of Huntington's and other neurodegenerative diseases (Keene et al., 2001, 2002; Rodrigues et al., 2003). Notably, TUDCA co-incubation inhibited $A\beta_{1-42}$- and $A\beta_{25-35}$-evoked apoptosis in PC12 neuronal cells (Ramalho et al., 2004; Viana et al., 2010). The compound did not affect Aβ aggregation as such (Viana et al., 2009), but was suggested to downregulate expression of connective tissue growth factor (CTGF) in hepatocytes (Castro et al., 2005), which suggests an approach to influence Aβ production more indirectly (Zhao et al., 2005).

Indeed, high hippocampal and neocortical expression of CTGF in *post-mortem* AD brains (Ueberham et al., 2003), and its co-localization with plaques and tangles suggest CTGF involvement in initiation and/or maintenance of AD neuropathology (Ueberham et al., 2003). Increased CTGF expression was accompanied by increased plaque formation (Ho et al., 2004; Zhao et al., 2005), and CTGF was shown to bind to the low-density lipoprotein-related protein receptor (LRP), which affects downstream amyloid deposition and tangle formation. Earlier work

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indicated that LRP-dependent signaling may trigger tau pathology by its link to *Wnt* signaling and glycogen synthase kinase 3β (Pinson et al., 2000).

We supplied TUDCA to the diet of APP/PS1 mice, an established AD mouse model (Radde et al., 2006), to investigate the preclinical therapeutic significance of the biochemical actions of this compound. We examined the functional benefits of TUDCA food supplementation in APP/PS1 mice using several hippocampus-dependent learning and memory tasks, and analyzed its effect on Aβ brain deposition. The use of transgenic AD models, which mimic at least a proportion of the behavioral and biochemical features of the disease, is well established in preclinical AD research (Hsiao et al., 1996; Oddo et al., 2003; Radde et al., 2006; Schindowski et al., 2006). The presently reported effects of TUDCA supplementation suggest a novel mechanistic route for AD therapeutics.

Methods and materials

Animals and feeding regime

The APP/PS1 mouse model was provided and licensed by Dr Mathias Jucker (Hertie Institute for Clinical Brain Research, Tübingen, Germany) and Dr Bart De Strooper (Laboratory for the Research of Neurodegenerative Diseases, University of Leuven, Belgium). Heterozygous male APP/PS1 mice from the Tübingen colony were crossbred at the Leuven animal facilities with female C57BL/6J mice from Elevage Janvier (Le-Genest-St-Isle, France). Offspring were genotyped using PCR on DNA isolated from tail biopsy as previously described (Radde et al., 2006). For behavioral testing, we used male APP/PS1 and wild type mice kept in standard animal cages under conventional laboratory conditions (12 h light/dark cycle, 22 °C), and *ad libitum* access to food and water. Experiments were conducted during the light phase of the activity cycle. All protocols have been reviewed and approved by the animal ethics committee of the University of Leuven.

The APP/PS1 mouse model has been shown to develop AD neuropathology at 2 months of age, whereas behavioral impairments occur from 8 months of age onwards (Radde et al., 2006). Therefore, we initiated TUDCA treatment at 2 months of age, when amyloid deposits have been shown to be still rather scarce in APP/PS1 mice, for a period of 6 months. APP/PS1 and wild type mice were randomly assigned to a diet containing 0.4% TUDCA (sodium salt; Prodotti Chimici e Alimentari S.p.A., Basaluzzo, Italy) (13 WT TUDCA and 15 APP/PS1 TUDCA) or regular food (10 WT control and 12 APP/PS1 control). Mice were handled for a week before assessing behavioral testing. Behavioral testing started at 8 months of age, where APP/PS1 control mice start to display memory deficits, and lasted for a month. Mice were on normal diet during behavioral testing. Weight was measured at the beginning of the behavioral testing and general activity was monitored during the course of experiments.

Morris water maze

Spatial learning abilities were tested in a standard hidden platform acquisition and memory retention version of the Morris water maze (Goddyn et al., 2006). The water maze consisted of a large circular pool (diameter 150 cm) filled with opacified water (26 ± 0.5 °C) and mice were trained for 10 days (twice 5 days with two days of rest in between) to find the circular escape platform (diameter 15 cm) that was hidden 1 cm beneath the water surface. Four trials starting from four different starting positions were performed each day with a trial interval of 30 min. When mice failed to find the platform within 2 min, they were guided to the platform and were left there for 15 s, before being returned to their cages. Latency to find the hidden platform, distance moved and swimming velocity were recorded with *Ethovision* (Noldus Bv, Wageningen, The Netherlands).

Acquisition trials were further analyzed to identify differential search strategies according to Brody and Holtzman (2006). Table 1 summarizes 8 different search strategies that were scored in these analyses. Such strategies ranged from proper *spatial strategies* to those that involved systematic scanning of the pool without actually relying on spatial information (*non-spatial strategies*), or those that merely consisted of repetitive loopings. The time each of these search strategies was maintained during the acquisition trials was measured by a researcher blinded to the experimental conditions. When different strategies were used during a particular acquisition trial, the predominant strategy was noted. Eventually, we analyzed the use of different search strategies during the course of the experiment.

To evaluate retention memory, probe trials were presented on days 6 and 11. During these probe trials, the platform was removed, and the swimming path was recorded during 100 s. Time spent in each quadrant was measured. We also visualized these swimming paths using a custom-made MATLAB protocol (Balschun et al., 2010; Van der Jeugd et al., 2011). Briefly, swimming paths of individual mice were placed on top of each other to create heat plots for every group. Color intensities (from blue to red) indicated relative presence in specific areas of the pool.

Social recognition

A social novelty and recognition task was adapted from Nadler et al. (Nadler et al., 2004), and described in detail elsewhere (Naert et al., 2011). Briefly, the setup consisted of three compartments divided by transparent Plexiglas walls with guillotine doors. A round holding cage (diameter 12 cm) was placed in each of the two outer compartments. The procedure consisted of three consecutive phases, between the phases the mouse was contained in the middle compartment. During the first phase (*acclimation phase*), mice were habituated to the apparatus for 5 min. In a second phase (*sociability phase*), an unfamiliar male mouse (S1) was introduced in one holding cage on one side while the other remained empty (−). Exploratory behavior (exploring and sniffing) towards S1 and the empty cage was recorded for 10 min. In the third phase (*social recognition phase*), a second unfamiliar mouse (S2) was placed in the formerly empty holding cage. Exploratory behavior towards both S1 and S2 was measured for 10 min. Exploratory behavior was defined as sniffing time towards the cage (nose oriented towards cage at a distance <2 cm). We calculated preference ratio ($Ratio_{Pref}$) as $Time_{S1}/(Time_{S1}+Time_{empty})$, and recognition ratio ($Ratio_{Rec}$) as $Time_{S2}/(Time_{S1}+Time_{S2})$. The position of S1 and S2 was counterbalanced between animals, and the apparatus was cleaned thoroughly with water after each trial.

Passive avoidance

Contextual fear learning was examined in a step-through box with a small illuminated compartment and a larger dark compartment fitted

Table 1
Summary of different search strategies mice can use to locate the hidden platform in the Morris water maze. These can be broadly classified as spatial, non-spatial or repetitive looping.

Main search strategy	Specific search strategy	Description search strategy
Spatial	Spatial direct	Mice swim to the platform in a straight line
	Spatial indirect	Mice swim to the platform with one small explorative loop
	Focal correct	Mice search for the platform in the correct quadrant
Non-spatial	Focal incorrect	Mice search for the platform in the wrong quadrant
	Scanning	Mice search for the platform in the center of the pool
	Random	Mice do not show preference to any part of the pool
Repetitive looping	Chaining	Mice search in the target annulus area
	Thigmotaxis	Mice display predontinant wall hugging behavior