The bile acid TUDCA and neurodegenerative disorders: an overview

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ABSTRACT

Bear bile has been used in Traditional Chinese Medicine for thousands of years due to its therapeutic potential and clinical applications. The tauroursodeoxycholic acid (TUDCA), one of the acids found in bear bile, is a hydrophilic bile acid and naturally produced in the liver by conjugation of taurine to ursodeoxycholic acid (UDCA). Several studies have shown that TUDCA has neuroprotective action in several models of neurodegenerative disorders (ND), including Alzheimer's disease, Parkinson's disease, and Huntington's disease, based on its potent ability to inhibit apoptosis, attenuate oxidative stress, and reduce endoplasmic reticulum stress in different experimental models of these illnesses. Our research extends the knowledge of the bile acid TUDCA actions in ND and the mechanisms and pathways involved in its cytoprotective effects on the brain, providing a novel perspective and opportunities for treatment of these diseases.

1. INTRODUCTION

Neurodegenerative disorders (ND) are devastating diseases characterized by progressive and irreversible neuronal dysfunction and death (Dugger & Dickson, 2017). The pathophysiological mechanisms of these diseases are diverse and involve distinct subgroups of neurons in specific areas of the brain (Ahmed et al., 2016), and consequently they can affect the behavior, cognition, metabolism and motor abilities (Gitler, Dhillon, & Shorter, 2017).

Alzheimer's disease (AD), Parkinson disease (PD) and Huntington disease (HD) are serious ND that affect people nowadays, and not only cause severe distress for patients and caregivers, but also result in a large socioeconomic burden (Wang, Gu, Masters, & Wang, 2017). Some of these disorders, such as AD and PD, are becoming increasingly prevalent, and this rise is, at least in part, due to the increase in life expectancy (Heemels, 2016), once elderly population has been increasing in recent years (Gitler et al., 2017; Hou et al., 2019).

Based upon the fact that the availability of an effective treatment for the millions of people who are diagnosed with ND is far from satisfactory, there is a crucial need to develop new and more efficient approaches treatment to combat these prevalent disorders.

Several strategies are currently being used to treat these illnesses, including bile acids. Since it has been shown for the first time that the systemic application of a bile acid provides neuroprotection (Keene et al., 2001), the effects of bile acids, more specifically the tauroursodeoxycholic acid (TUDCA), has been studied intensively in ND. Herein, an overview will be provided on the bile acid TUDCA effects in

Alzheimer’s, Parkinson's and Huntington's diseases, once the conditions just mentioned represent a major threat to human health.

2. THE TAUROURSODEOXYCHOLIC ACID – TUDCA

Initially considered to be detergent molecules with an important role in lipid digestion, bile acids have proven to have importance in a myriad of biological processes (Marin, Macias, Briz, Banales, & Monte, 2015). Specifically, they have an important role as regulatory molecules, interacting with many cellular receptors to modulate important signaling pathways (Hylemon et al., 2009). Their role in the liver and gastrointestinal system is well-established, and has been previously reviewed (Hylemon et al., 2009). Essentially, by interacting with specific intracellular and membrane receptors, bile acids are capable of modulating important pathways like c-jun n-terminal kinases (JNK) 1/2, extracellular signal-regulated kinases (ERK) 1/2 and AKT, which in turn results in the regulation of the synthesis and metabolism of lipids and glucose (Dent et al., 2005; Hylemon et al., 2009).

The bile acid TUDCA is a taurine conjugate of ursodeoxycholic acid (UDCA). Human liver is able to synthesize from cholesterol only primary bile acids, such as chenodeoxycholic acid (CDCA) and cholic acid (CA) (Kusaczuk, 2019). The secondary bile acid UDCA is produced exclusively by intestinal microbiota, through the epimerization of hydroxyl groups of CDCA by intestinal bacteria (Daruich, Picard, Boatright, & Behar-Cohen, 2019; Lepercq et al., 2004). Once produced, UDCA is directed to the liver with enterohepatic circulation and is then conjugated with taurine to form TUDCA (Kusaczuk, 2019; Li & Chiang, 2014), effect that reduces its toxicity and increases its solubility (Chiang, 2009).

Amongst the multiple bile acids with regulatory functions, the TUDCA has been the focus of much attention in the past years. The bile acid TUDCA is permeable to the blood-brain barrier and have a low toxicity profile (Cortez & Sim, 2014; Vang, Longley, Steer, & Low, 2014). In addition, UDCA, its precursor, is approved by the U.S. Food and Drug Administration (FDA) as a medication for cholestatic liver diseases (Amaral, Viana, Ramalho, Steer, & Rodrigues, 2009; Bahar, Wong, Liu, & Bowlus, 2018; Beuers, Boyer, & Paumgartner, 1998; Lazaridis, Gores, & Lindor, 2001). TUDCA has been shown to have a potent ability to combat endoplasmic reticulum (ER) stress, which is an important step in many cases of cellular dysfunction (Vang et al., 2014). Thus, TUDCA seems to be an important signaling molecule for many cellular functions in multiple tissues (Vang et al., 2014; Vettorazzi et al., 2016). However, the signaling pathways and the molecular mechanisms by which this bile acid acts are still not fully understood, delaying translation to the clinical setting.

As a signaling molecule, TUDCA differs from most bile acids. Due to its preferential interactions with membrane receptors – once this acid is more hydrophilic in nature (Chiang, 2013; Sepe et al., 2014) – it mainly acts through membrane receptors Takeda G protein receptor 5 (TGR5), sphingosine-1-phosphate receptor-2 (S1PR2) and α5β1-Integrin. TGR5 is coupled to a stimulatory G-protein, and plays important roles in cell signaling pathways, such as nuclear factor κB (NF-κB), AKT and ERK. TGR5 agonists may be potential compounds for treatment of metabolic, inflammation and digestive disorders (Guo, Chen, & Wang, 2016). Like TGR5, S1PR2 is also a G-protein-coupled receptor and its activation also leads to the activation of ERKs and AKT pathways (Kiriyama & Nochi, 2019). The integrin α5β1 is a heterodimeric transmembrane receptor that mediate cell– extracellular matrix (ECM) and cell–cell adhesion events (Wu & Reddy, 2012). Integrins are involved in synaptic plasticity in

neurodegenerative conditions (Wu & Reddy, 2012), and it has been reported to induce kinase activity in response to TUDCA (Gohlke, Schmitz, Sommerfeld, Reinehr, & Häussinger, 2013). The response of this receptor also involves the activation of the AKT and ERK signaling pathway (Brakebusch, Bouvard, Stanchi, Sakai, & Fässler, 2002).

On the other hand, it has been known that TUDCA evokes cellular effects after entering the hepatocyte without affecting extracellular membrane/receptor interactions (Gohlke et al., 2013). TUDCA can be transported into the cell via Na^+/taurocholate co-transporter peptide (NTCP), a hepatocyte-specific solute carrier, and activate the α5β1-Integrin by interacting with this receptor inside the hepatocyte (Beuers, 2013; Gohlke et al., 2013). This effect generates phosphorylation and activation of ERK1/2, epidermal growth factor receptor (EGFR), and other downstream events (Gohlke et al., 2013). Furthermore, after internalization, TUDCA can also activate nuclear receptors, such as farnesoid X receptor (FXR), glucocorticoid receptor (GR) (Kiriyama & Nochi, 2019), and mineralocorticoid receptor (MR) (Kiriyama & Nochi, 2019; Solá et al., 2006).

Considering that most effects of TUDCA are dependent on the activation of TGR5, S1PR2 and α5β1-Integrin, (McMillin & DeMorrow, 2016; Yanguas-Casás, Barreda-Manso, Nieto-Sampedro, & Romero-Ramírez, 2017) and the three of them have already been identified in the brain (Kim et al., 2015; Shapiro, Kolodziejczyk, Halstuch, & Elinav, 2018; Wu & Reddy, 2012), this bile acid can act centrally. TUDCA signaling in the brain is summarized in Figure 1.

Therefore, of particular interest to this review, TUDCA has been shown to have beneficial effects in AD, PD and HD. These disorders share common crucial feature: accumulation of protein aggregates in the brain (Soto & Pritzkow, 2018). Different proteins are implicated in each disease: amyloid-β (Aβ) and TAU in AD, α-synuclein in

PD and huntingtin in HD. Many evidences have indicated that protein misfolding and aggregation, leading to ER stress, are central factors of pathogenicity in ND (Shacham, Sharma, & Lederkremer, 2019). Considering the ability of TUDCA to efficiently mitigate the accumulation of toxic protein aggregates and ER stress in different experimental models of ND (Cortez & Sim, 2014), the use of this bile acid in the treatment of these conditions is promising. TUDCA's actions involved in delaying neurodegeneration in AD, PD and HD will be explored in the next sections.

3. ALZHEIMER'S DISEASE

Alzheimer's disease is a neurodegenerative disorder and the most common of the late-life dementias (Walsh & Selkoe, 2004). AD pathogenesis is complex, involving abnormal Aβ metabolism and gradual accumulation (Selkoe, 2001; Wang et al., 2017), aberrantly hyperphosphorylated TAU protein (Rudrabhatla, Jaffe, & Pant, 2011), neuroinflammation (Heneka et al., 2015), and other pathological events, resulting in atrophy of the hippocampal formation and cerebral cortex (Walsh & Selkoe, 2004). Once these brain regions are important for memory, neuronal death culminates in progressive memory impairment, cognitive disabilities and altered behavior (Selkoe, 2001).

AD development is probably multifactorial, and aging is the greatest risk factor for its onset. Just a small percentage of AD patients have the inherited form of the disease, with early onset in life and related to a specific hereditary mutation (Kozlov, Afonin, Evsyukov, & Bondarenko, 2017). The average duration of AD is around 8–10 years, although asymptomatic phase can be extended by more than 20 years. Most patients with AD (>95%) present the sporadic form of the disease, which has a mean age of onset of 80 years old. Although the main cause of Alzheimer's is still unknown, it is

believed that one of the causes is an unbalance between Aβ production and clearance (Masters et al., 2015).

Several targets have been used for AD treatment since it was described by Alois Alzheimer in 1907. In this way, bile acids, especially the bile acid TUDCA, has shown a great therapeutic potential for the treatment of this disease.

One of the first signs that the bile acid TUDCA could have beneficial effects in AD came from studies performed in 2003, in which TUDCA inhibited Aβ-induced apoptosis in primary culture of rat cortical neurons (Solá, Castro, Laires, Steer, & Rodrigues, 2003). Since then, both *in vitro* and *in vivo* models have supported TUDCA as an important candidate in AD treatment.

Apoptosis is frequently found in AD pathology (Ramalho, Viana, Low, Steer, & Rodrigues, 2008), and mitochondria have a prominent role in apoptosis, which is mediated through permeabilization of the mitochondrial membrane, impairment of Ca^{2+} homeostasis, and release of proapoptotic molecules (Kroemer & Reed, 2000). TUDCA actions in preventing apoptosis by several stimuli in neuronal cells have been intensely studied. The treatment with 100 μM of TUDCA for 12 h can significantly decrease Aβ peptide-associated apoptosis in cortical neurons. The cell death inhibition triggered by TUDCA involves the PI3K signaling cascade that suppresses Bax translocation (Solá et al., 2003). TUDCA also abrogates Aβ-induced apoptosis in PC12 neuronal cells (Fonseca, Nunes, & Rodrigues, 2012; Ramalho et al., 2004; Viana, Ramalho, Nunes, Steer, & Rodrigues, 2010; Viana, Steer, & Rodrigues, 2011), by 1) modulating E2F-1 induction, p53 stabilization and Bax expression, resulting in inhibition of E2F-1/p53 apoptotic pathway; 2) modulating the expression of Bcl-2 family elements (Ramalho et al., 2004); 3) preventing the caspase-12 activation (Viana et al., 2011); 4) inhibiting

JNK early activation and nuclear translocation (Viana et al., 2010); and 5) preventing anti-apoptotic ΔNp63 degradation, through changes of c-Jun levels (Fonseca et al., 2012). Considering the role of apoptosis in Aβ-induced toxicity, the combined effects of TUDCA in attenuating apoptosis could weigh on the delay in the neurodegenerative process in individuals with AD.

TUDCA's action was also reported in mutant neuroblastoma cells that presented increased Aβ production and aggregation, and p53-mediated apoptosis (Ramalho et al., 2006). In this study, it was observed that neuroblastoma cells exposed to 100 μm of TUDCA for 12h presented reduced nuclear fragmentation and caspase 2 and 6 activation, decreasing caspase-dependent apoptosis. In addition, TUDCA treatment also reduced p53 protein expression, and modulated the Bcl-2 and Bax proteins (Ramalho et al., 2006), suggesting that the downregulation of p53 and its downstream targets decrease the transcriptional activation of the pro-apoptotic proteins, preventing the cell death program from occurring. Corroborating with these findings, TUDCA also inhibited apoptosis induced by the vasculotropic E22Q mutant of the amyloid-β (AβE22Q), in primary human cerebral endothelial cells (Viana et al., 2009), reducing the cytochrome c release from mitochondria and Bax translocation.

A possible mechanism by which TUDCA inhibits Aβ-induced apoptosis is through the nuclear steroid receptor (NSR) (Solá et al., 2006). Incubation with Aβ slightly increased MR levels in primary rat cortical neurons, while treatment with TUDCA further enhanced MR expression. TUDCA interacts with a specific region of MR ligand binding domain and dissociates MR from heat shock protein 90, its cytosolic chaperone. Thus, a complex of TUDCA and MR translocates into the nucleus, modulating NSR transactivation and ultimately inhibiting Aβ-induced apoptosis. Furthermore, when MR siRNA is used the antiapoptotic effect of TUDCA is abolished (Solá et al., 2006).

Cytosolic calcium has also been implicated as a proapoptotic second messenger involved in both triggering apoptosis and regulating caspases (Nicotera & Orrenius, 1998; Wertz & Dixit, 2000; Xie et al., 2002). In the liver, TUDCA caused concentration-dependent decreases in intracellular calcium (Gleeson, Murphy, & Dowling, 1990), modulating calcium homeostasis (Beuers, Nathanson, & Boyer, 1993; Beuers, Nathanson, Isales, & Boyer, 1993; Gleeson et al., 1990; Xie et al., 2002) and preventing apoptosis, by blocking a calcium-mediated apoptotic pathway as well as caspase-12 activation (Xie et al., 2002). Since TUDCA can inhibit apoptosis by ameliorating ER stress through modulating intracellular calcium (Xie et al., 2002), calcium signaling should exert an important role in these events. Although the effects of TUDCA on calcium signaling in neural cells are poorly understood, these findings suggest that TUDCA's actions in modulating calcium homeostasis may also play an important role in the anti-apoptotic effect during neurodegeneration.

At the synaptic level, TUDCA modulates synaptic deficits induced by Aβ in primary cortical and hippocampal neurons. 12 h incubation with 100 μM of TUDCA resulted in inhibition of the postsynaptic marker, postsynaptic density-95 (PSD-95) downregulation; decrease in spontaneous miniature excitatory postsynaptic currents (mEPSCs) frequency; and increase in the number of dendritic spines (Ramalho et al., 2013). Cortical and hippocampal synapse density is early reduced during AD pathogenesis, and synaptic loss is the best pathological correlate of cognitive impairment in AD (Ramalho et al., 2013). TUDCA's actions at a synaptic level suggest that their protective role goes beyond its capacity to modulate neuronal death.

Considering that the accumulation of misfolded and aggregated proteins is common in AD, specific markers for unfolded protein response (UPR) activation are increased in AD brain tissue (Scheper & Hoozemans, 2015). It has been described that TUDCA

inhibits UPR in human neuroblastoma cell line, previously treated with 2-deoxy glucose UPR inducer, preventing the TAU hyperphosphorylation (van der Harg et al., 2014), suggesting that the inhibition of UPR by TUDCA, as chemical chaperone, could be a putative target for therapeutic intervention in AD.

In APP/PS1 mice, an experimental model of AD, a 6-month treatment with 0.4% of TUDCA in diet prevented Aβ plaque accumulation in the brain (Lo, Callaerts-Vegh, Nunes, Rodrigues, & D'Hooge, 2013; Nunes et al., 2012). This treatment reduced the activation of astrocytes and microglia, as well as increased immunoreactivity of MAP2, a marker of neuronal integrity, in the hippocampus (Nunes et al., 2012). Moreover, an improvement in the spatial, recognition and contextual memory was also observed in APP/PS1 mice after this treatment (Lo et al., 2013; Nunes et al., 2012).

Studies suggest that neuroinflammation plays a causal role in AD pathogenesis (Heneka et al., 2015). There are some evidences that the bile acid TUDCA has anti-inflammatory properties in mice model of acute neuroinflammation and glial cells treated with proinflammatory stimuli (Hassan, Bhalla, El Regal, & A-Kader, 2014). In this study, TUDCA treatment reduced hippocampal microglial activation and vascular cell adhesion molecule 1 (VCAM-1) production in mice, inhibited iNOS and nitrite production in cultured glial cell, and reduced microglial cell migratory capacity. All these effects were possibly a result of nuclear factor NFκB activity inhibition observed with TUDCA administration. Once neuroinflammation is strongly associated with accelerated AD progression, the anti-inflammatory properties of TUDCA further highlight its therapeutic potential in that condition.

It has been reported that TUDCA treatment improves synaptic deficits, reducing synaptic loss in 7 months old APP/PS1 mice (Dionísio et al., 2015). TUDCA also inhibited the impaired PSD-95 reactivity and protein levels in the hippocampus in 8

months old APP/PS1 mice (Ramalho et al., 2013), evidencing the properties of the bile acid studied here as an important agent in keeping synapse efficiency, even in pathological conditions.

Studies show that the treatment with TUDCA interferes with Aβ production (Dionísio et al., 2015; Nunes et al., 2012). Aβ is derived from the APP, through sequential cleavages by β- and γ-secretase enzyme activities, which characterizes the amyloidogenic APP processing pathway (Chow, Mattson, Wong, & Gleichmann, 2010; Zhang, Thompson, Zhang, & Xu, 2011). This pathway results in the generation of soluble APP-β fragment (sAPPβ), carboxyterminal fragments (CTFs) and finally Aβ (Chow et al., 2010). A decrease in the production of CTFs and sAPP-β is observed in TUDCA-treated APP/PS1 mice. These changes culminate in reduced Aβ1–40 and Aβ1–42 levels and amyloid plaque burden in hippocampus and frontal cortex, suggesting an overall modulation in amyloidogenic APP processing when TUDCA is administrated (Dionísio et al., 2015; Nunes et al., 2012).

Apolipoprotein E type 4 (ApoE4) allele is the major known genetic risk factor for AD (Corder et al., 1993; Mahley, Weisgraber, & Huang, 2009). It has been reported that exogenous ApoE4 increases Aβ production (Ye et al., 2005), lysosomal leakage and apoptosis (Ji et al., 2002) in neuronal cells. TUDCA administration (2 mM) in cultured macrophages with ApoE4 improved efferocytosis, reduced cell death, and attenuated LPS- and oxLDL-induced apoptosis (Cash et al., 2012), probably through the reduction of ER stress. In APP/PS1 mice, TUDCA decreased the gene expression of ApoE in hippocampus and frontal cortex, as well as significantly reduced the gene expression of other lipid-metabolism mediators involved in Aβ production and accumulation (Nunes et al., 2012). Thus, these modulations could be responsible for the reduction of Aβ

production found in these mice, since evidence suggests that abnormal lipid-metabolism is associated with a raised risk of AD pathogenesis (Lesser, 2012).

Finally, it was recently shown that 10-days of TUDCA treatment (300 mg/kg) improves glucose metabolism in streptozotocin-induced AD mice model (Zangerolamo et al., 2020), by improving glucose tolerance and insulin sensitivity, reducing fasting and feeding glycemia, enhancing pancreatic islet mass and β-cell area, and increasing glucose-stimulated insulin secretion. Impaired glucose tolerance and insulin resistance are often found in AD patients (Mittal & Katare, 2016) and mouse models of AD (Clarke et al., 2015; Macklin et al., 2017; Shinohara & Sato, 2017). Besides that, insulin resistance is considered one of the major risk factors associated with AD (Bedse, Di Domenico, Serviddio, & Cassano, 2015; Hildreth, Van Pelt, & Schwartz, 2012). The study by Zangerolamo and colleagues shows that the beneficial actions of TUDCA involved in delaying neurodegeneration in AD are not limited to the central nervous system, since the effects of TUDCA on peripheral tissues will also have an impact on the mitigation of the main neuromarkers of AD.

Taken together, both *in vitro* and *in vivo* results show the effectiveness of TUDCA in decreasing apoptosis, attenuating Aβ production and deposition, TAU hyperphosphorylation, glial activation, neuroinflammation, and loss of synaptic function. These evidences, allied to its low toxicity and brain bioavailability, point TUDCA as a promising therapeutic intervention to attenuate AD progression.

4. PARKINSON'S DISEASE

PD is the second most common neurodegenerative disease, characterized by the progressive death of dopaminergic neurons in substantia nigra region of the brain and intracellular deposition of α-synuclein protein, generating the Lewy Bodies (Beitz,

2014; Poewe et al., 2017), resulting in impairment of motor control, such as bradykinesia, rigidity and rest tremor (Davie, 2008).

Symptoms manifest slowly and gradually over time, with an asymptomatic phase, followed by substantia nigra damage and neocortex impairment (Davie, 2008). When PD symptoms start to appear, about 50-70% of the substantia nigra neurons have already degenerated (Postuma, Gagnon, & Montplaisir, 2010). Non-motor symptoms of PD include cognitive impairment, usually 10 years or more after the onset of motor symptoms (Davie, 2008), behavioral changes and sleep disturbances (Beitz, 2014).

The heritable forms of PD is uncommon and represent only 5–10% of all cases (Poewe et al., 2017). Most cases are age-dependent, affecting 2-3% of the world's population over 65 years (Poewe et al., 2017). Although PD has no cure, there are several therapies that can improve the patient's quality of life (Connolly & Lang, 2014).

Drug therapy in Parkinson’s patients seek to reduce dopamine (DA) deficit (Beitz, 2014) and are composed mainly of DA agonists and agents that block DA degradation (Connolly & Lang, 2014). Moreover, surgical approaches are available to treat early and late complications of this illness (Davie, 2008).

Several studies have been pointing mitochondrial dysfunction as a key element in the pathogenesis of PD (Poewe et al., 2017). Mitochondria play important roles in activating apoptosis in mammalian cells, thereby exhibiting major changes in their structure and function (Eckert et al., 2003). In this sense, most studies that evaluate the effects of TUDCA treatment in PD models focus their attention on decreasing apoptosis.

One of the first studies to show that TUDCA could block apoptotic pathways in DA neurons was done by Duan and colleagues (Duan, Rodrigures, Zhao, Steer, & Low,

2002). They showed that the application of TUDCA facilitates the survival of DA neurons *in vitro* and *in vivo* conditions. Seven days *in vitro*, DA neurons presented dramatic cell loss, which was prevented by TUDCA treatment (50 μM was found to be optimal). Moreover, it was observed in the TUDCA-treated group: an increase in the number of tyrosine hydroxylase positive neurons, used as a marker for dopamine, norepinephrine, and epinephrine-containing neurons (Weihe, Depboylu, Schütz, Schäfer, & Eiden, 2006); and a reduction in the number of apoptotic cells.

Based on these beneficial effects of TUDCA on DA neurons, it was evaluated whether the supplementation of TUDCA to cell suspensions prior to transplantation could lead to enhanced survival of nigral grafts, once it was previously observed that 80-95% of grafted DA neurons die following transplantation (Brundin & Björklund, 1987). Supplementation with 50 μM of TUDCA to cell suspensions prior to transplantation enhanced the survival and function of nigral transplants in a rat model of PD (Duan et al., 2002). TUDCA treatment also significantly reduced apoptosis in ventral mesencephalic tissue cultures and within the transplants (Duan et al., 2002). Thus, the reduced death of neural transplants by previous TUDCA treatment brought to light how relevant the bile acid could be for possible therapies in PD patients. This reduction also suggests that TUDCA exerts beneficial effects on DA neuron survival mainly through antiapoptotic mechanisms. The study provided further supportive evidence to the notion that apoptosis may be the main contributor to the loss of DA neurons in PD.

TUDCA's anti-apoptotic actions have also been evaluated in the genetic PD *Caenorhabditis elegans* model. Ved and colleagues showed that a combined pharmacological approach with TUDCA and D-β-hydroxybutyrate activates mitochondrial complex II and inhibits apoptosis induced by rotenone in the presence of

PD-related genetic changes, assuming that TUDCA acts in the worm to partially rescue mitochondrial dysfunction (Ved et al., 2005).

In human neuroblastoma SH-SY5Y cell line, TUDCA prevents both 1-methyl-4-phenylpyridinium and α-synuclein-induced oxidative stress, through the activation of nuclear factor erythroid 2 related factor 2 (Nrf2) (Moreira et al., 2017). Nrf2 is the master regulator of cellular redox status, preventing the initiation of cell death programs (Benarroch, 2017). Once cell death in PD is often associated with increased oxidative stress, strategies to increase the levels of antioxidant enzymes could produce a positive effect. Under oxidative stress, Nrf2 activates the transcription of antioxidant enzymes Glutathione peroxidase (GPx) and Heme oxygenase-1 (HO-1). The Nrf2 signaling pathway was evaluated in TUDCA-treated PD mice model, and it was observed that TUDCA increased the expression of Nrf2 and Nrf2 stabilizer DJ-1, as well as the Nrf2 downstream antioxidant enzymes. Furthermore, in these treated mice, it was also found that TUDCA enhanced GPx activity in midbrain and striatum (Moreira et al., 2017) and increased the levels of antioxidant enzymes GPx and HO-1 in the cortex (Mendes et al., 2019). Overall, TUDCA is promising to mitigate oxidative stress in different models of PD, and it is plausible that an important part of TUDCA protective effects should be mediated by Nrf2 activation.

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is one of the most commonly used compound to induce PD in mice, since it causes degeneration of nigrostriatal dopaminergic neurons (Huang et al., 2017; Vang et al., 2014). The effects of TUDCA in this mice model have already been studied.

Pre-treatment with TUDCA (50 mg/kg body weight) significantly reduced neurodegeneration of the nigral dopaminergic neurons caused by MPTP, as well as

reduced dopaminergic fiber loss (Castro-Caldas, Carvalho, Rodrigues, Henderson, Wolf, Rodrigues, et al., 2012).

Considering that JNK plays an important role in dopaminergic neuronal death in substantia nigra (Castro-Caldas, Carvalho, Rodrigues, Henderson, Wolf, & Gama, 2012), the effect of TUDCA on that target was evaluated. Indeed, TUDCA prevented MPTP-induced JNK phosphorylation in the midbrain and striatum of glutathione S-transferase pi (GSTP) null PD mice model (Castro-Caldas, Carvalho, Rodrigues, Henderson, Wolf, Rodrigues, et al., 2012).

MPTP toxicity is also associated with higher reactive oxygen species (ROS) generation, which in turn results in apoptosis. TUDCA treatment prevented the production of MPTP-dependent ROS in GSTP null mice (Castro-Caldas, Carvalho, Rodrigues, Henderson, Wolf, Rodrigues, et al., 2012). In addition, survival pathway activated by TUDCA involves pro-survival AKT signaling, through activating downstream NF-κB pathway in GSTP null mice midbrain (Castro-Caldas, Carvalho, Rodrigues, Henderson, Wolf, Rodrigues, et al., 2012). The activation of the AKT pathway by TUDCA had been previously observed (Castro, Solá, Ramalho, Steer, & Rodrigues, 2004; Rodrigues et al., 2003; Solá et al., 2003), and the mechanisms of activation of this pathway could be mediated by the activation of TGR5, S1PR2 or α5β1-Integrin receptors, which, in turn, would result in attenuation of the deleterious effects of MPTP in GSTP null mice.

Inflammatory responses manifested by glial reactions, increased expression of inflammatory cytokines, and other toxic mediators derived from activated glial cells are currently recognized as prominent features of PD and may further propel the progressive loss of nigral DA neurons (Tufekci, Meuwissen, Genc, & Genc, 2012). TUDCA treatment has shown efficiency in attenuating inflammation, by reducing the

levels of cortical astro- and microgliosis markers: glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba-1), respectively. TUDCA also increased the protein content of anti-inflammatory protein Annexin-A1 (ANXA-1) and reduced the protein content of proinflammatory cytokine Interleukin-1beta, in MPTP-treated mice. Corroborating *in vivo* data, the treatment with 100 μM of TUDCA also increased the ANXA-1 expression and secretion, in mouse microglia cell line BV2 (Mendes et al., 2019). These data suggest a link between inhibition of neuroinflammation and neuroprotection by TUDCA and open opportunities for further works to be carried out, in order to have a fully understanding of the molecular mechanisms involved in TUDCA's anti-inflammatory properties in PD.

The benefits of TUDCA treatment in MPTP-treated mice it was also performed by Rosa and colleagues (Rosa et al., 2017). In this study, it is shown that mice pre-treated with TUDCA presented increased levels of full-length PTEN-induced putative kinase 1 (PINK1), as well as phosphorylated Parkin, in the brain. PINK1 has been described, together with Parkin, to mediate mitochondria degradation by mitophagy; and failure of mitophagic process leads to accumulation of damaged mitochondria, resulting in increased oxidative stress and cell death (Geisler et al., 2010; Rosa et al., 2017). The TUDCA-dependent mitoprotective effects have also been observed in primary mouse cortical neurons and neuroblastoma cell line SH-SY5Y (Rosa et al., 2017). PINK1 can act as a molecular sensor of damaged mitochondria, and the mitophagic process is stimulated when active PINK1 accumulates on the mitochondrial surface (Jin & Youle, 2012). When the mitochondria are damaged, PINK1 activates Parkin and ubiquitin by phosphorylation, thus, phospho-Parkin undergoes a closed-to-open conformational change, binds to phospho-ubiquitin, and becomes fully active (Eiyama & Okamoto, 2015). Activated Parkin then builds ubiquitin chains on damaged mitochondria to tag

them for degradation in lysosomes (Bingol & Sheng, 2016). These findings suggest that the mitophagic process may account for the unraveling TUDCA antioxidant potential in experimental models of PD, and its neuroprotective effects occur through the modification of PINK1/Parkin-mediated mitophagy.

Ultimately, the effects of TUDCA in motor symptoms in a mouse model of PD were described by Rosa and colleagues (Rosa et al., 2018). It has been demonstrated that TUDCA administration (50 mg/Kg for 3 days) ameliorated motor performance and symptoms typical of PD, such as spontaneous activity, ability to initiate movement and tremors. Once modulation of glial activation and neuroinflammation preceded or coincided with the manifestation of MPTP-induced damage in the striatum, the improvement of TUDCA-dependent motor symptoms must involve these processes, since modulation of these phenomena were observed after treatment with this bile acid.

Taken together, the results show that the bile acid TUDCA has neuroprotective effects in both *in vitro* and *in vivo* experimental models of PD. The ability of TUDCA in attenuating mitochondrial dysfunction, ROS production and neuroinflammation, as well as inhibiting multiple proteins involved in apoptosis and upregulation of cell survival pathways, points this bile acid as a promising therapeutic agent to be implemented in the treatment of PD.

5. HUNTINGTON'S DISEASE

HD is an inherited neurodegenerative disorder, caused by a CAG trinucleotide repeat expansion within exon 1 of the IT15 gene, also called HTT, the gene that encodes the huntingtin (HTT) protein. The CAG repeat is translated into a long polyglutamine (polyQ) sequence. HD is associated with 36 or more of these repetitions (Bates et al., 2015). The HTT protein exhibits toxic properties that cause synaptic dysfunction and

neuronal death, mainly in medium spiny neurons of the striatum (Roos, 2010; Smith, Brundin, & Li, 2005).

As observed in AD and PD, protein aggregates formation is also found in HD (Bates et al., 2015; Davies et al., 1997). Mutant HTT accumulates and form insoluble intracellular inclusions, a common pathological hallmark of HD (Keene et al., 2002). It has been suggested that these aggregates can impair the ubiquitin-proteasome system, neurotransmission and gene expression modulation, by binding to CREB-binding protein (Smith et al., 2005).

Patients with HD gradually develop abnormal motor dysfunction (most typically chorea), cognitive decline, psychiatric disturbances and personality changes (Bates et al., 2015; Roos, 2010; Smith et al., 2005). Mean age at onset of symptoms is 30-50 years, though in some cases symptoms can start before 20 years of age, with behavior disorders and learning problems, but without chorea (Roos, 2010; Smith et al., 2005). In this case, the pathology is called Juvenile Huntington's disease. Mostly of HD cases progress to death in around 15–20 years after the onset of symptoms (Smith et al., 2005).

Although HD has no cure or ways to be avoided or slowed, this disease is not untreatable. The treatments focus in mitigate the symptoms, through medical and non-medical procedures, in search of to improve the quality of life of the patient.

As observed in AD e PD, mitochondrial dysfunctions also plays a major role in HD pathogenesis (Carmo, Naia, Lopes, & Rego, 2018). DNA fragmentation, characteristic of apoptosis, is elevated in HD neostriatum and is positively correlated with CAG repeat length. Moreover, caspases activation, which is crucial for the initiation and execution of apoptosis, is elevated in HD brains (Keene et al., 2001).

The toxin 3-nitropropionic acid (3-NP) is an irreversible inhibitor of mitochondrial succinate dehydrogenase, and can induce oxidative damage and impaired antioxidant defense enzymes in the brain, leading to oxidized proteins in the striatum and massive loss of striatal neurons (Gao et al., 2015). This toxin has been used to explore the molecular mechanisms of cell death associated with mitochondrial dysfunction in HD.

The treatment with TUDCA exhibited a significant reduction in apoptosis in a 3-NP rat model of HD, as well as preserved striatal mitochondria morphology and reduced striatal lesion volumes (Keene et al., 2001). It was also observed an improvement in sensorimotor and cognitive deficits associated with 3-NP toxicity. In cultured striatal cells, TUDCA treatment prevented 3-NP-mediated neuronal death (Keene et al., 2001). The study performed by Keene and colleagues was the first to show that systemically administered bile acid can reach the brain and perform neuroprotective functions, pointing bile acids as a potential therapeutic in the treatment of certain neurological diseases.

The mechanism by which TUDCA inhibits apoptosis in 3-NP neurotoxicity is not fully elucidated. A study performed by Rodrigues and colleagues (Rodrigues et al., 2000) showed that isolated mitochondria from rat brain incubated with 3-NP and 500 μM TUDCA presented reduced mitochondrial swelling and mitochondrial release of cytochrome c. Pretreatment with TUDCA also inhibited ROS production by 3-NP, and decreased caspase 3 activity and nuclear fragmentation. An important finding of this work was to show that TUDCA treatment results in decreased levels of Bax protein in the cytosol and increased proportionately in mitochondria, followed by inhibition of mitochondrial depolarization, suggesting that TUDCA modulates 3-NP-induced apoptosis by stabilizing the mitochondrial membrane, thus preventing cytochrome c release and activation of apoptotic pathways (Rodrigues et al., 2000). These

modulations by TUDCA treatment in prevent apoptosis were similar to found in AD mutant neuroblastoma cells (Ramalho et al., 2006), confirming the beneficial effect of TUDCA on preventing apoptosis in ND models.

The treatment with 500 mg/kg of TUDCA also generated neuroprotective effects in the R6/2 transgenic mice model of HD (Keene et al., 2002). In this treatment, TUDCA reduced striatal apoptosis and atrophy. The size and number of ubiquitinated neuronal intranuclear inclusions (NII) were also reduced. About the motor abilities, the TUDCA-treated mice exhibited significantly improvement in locomotor and sensorimotor deficits.

Although further intensive investigations are still necessary to provide evidence for TUDCA activity in HD suffering individuals, the neuroprotective effects of TUDCA in transgenic and pharmacologically-induced HD mice model, suggest that this bile acid can provide a novel and effective treatment in patients with HD.

6. CLINICAL APPROACHES

Despite several promising reports demonstrate favorable effects of TUDCA in models of neurodegeneration, these benefits in a clinical setting remains poorly explored.

Currently, there is one registered clinical trial with TUDCA in AD, in the United States (Clinical Trials registration: NCT03533257). The proposed study will be randomized, double-blind, multi-site, and placebo-controlled in volunteers with late mild cognitive impairment (MCI) or early dementia due to AD. The study was designed to evaluate the safety, tolerability, drug target engagement and neurobiological effects of treatment with AMX0035 (combination therapy of TUDCA and Sodium Phenylbutyrate) during 24 weeks.

There are also three clinical trials registration with TUDCA precursor bile acid, UDCA, in patients with PD and HD. The former, entitled “Brain Bioenergetics in Parkinson's Disease and Response to Repeated Oral UDCA Treatment” (Clinical Trials registration: NCT02967250) is being developed in the United States, and aims to understand the bioenergetic impairments that underlie PD and evaluating treatments that may improve mitochondrial dysfunction that is present in PD patients. The hypothesis is that repeated oral dosing of UDCA will result in increased brain ATP levels in individuals with PD. The second one, entitled “Trial of Ursodeoxycholic Acid (UDCA) for Parkinson's Disease: The "UP" Study” (Clinical Trials registration: NCT03840005), is a British study, focusing on assessing the safety and tolerability of UDCA in patients with PD, in order to slow down the worsening of the disease. And the later, entitled “Ursodiol in Huntington's Disease” (UDCA-HD) (Clinical Trials registration: NCT00514774), takes into account the anti-apoptotic effects that TUDCA had on experimental HD models, both in cells and in rodents, to establish a preliminary safety and tolerability profile of the drug in patients with HD, as well as to evaluate whether this treatment result in measurable levels of its bile acid metabolites in serum and cerebrospinal fluid (CSF) at standard oral doses. Whereas in healthy humans administered ursodiol (commercially-available exogenous form of UDCA) (15 mg/kg/day) for 3 weeks, biliary and duodenal bile acid concentrations of UDCA and its conjugates (glycoursodeoxycholic acid, GUDCA and TUDCA) increased by 40% compared to baseline (Dilger et al., 2012), these studies will also be relevant for understanding the safety of TUDCA in humans.

It is worth mentioning that although a clinical study directly comparing the effectiveness of TUDCA in relation to UDCA in patients with some type of ND has not been carried out yet, after oral administration, TUDCA is better absorbed by intestine,

as it is fully ionized and water soluble at the various pH value (Pan et al., 2013). Besides that, TUDCA undergoes less biotransformation than UDCA (Invernizzi et al., 1999; Pan et al., 2013). Such evidences suggest that TUDCA has significant advantages over UDCA, which may be beneficial for the clinical setting.

Other ongoing registered clinical trials of TUDCA and UDCA in neurodegeneration are summarized in Table 1, none of which has results available to date.

Although there are still no human data on the therapeutic use of TUDCA in AD, PD or HD, clinical trials indicated potential effectiveness, safety, good absorption after oral administration and penetration into the CSF, and overall good tolerance of TUDCA and UDCA in patients with Amyotrophic Lateral Sclerosis (ALS) (Elia et al., 2016; Min et al., 2012; Parry et al., 2010). In addition, it has recently been reported that the combination of Sodium Phenylbutyrate and TUDCA resulted in slower functional decline than placebo in ALS patients, based on the therapeutic capacity of these compounds to attenuate the toxicity from ER stress and to recover mitochondrial bioenergetic deficits (Paganoni et al., 2020). The results of the ongoing studies will be fundamental to support the findings of pre-clinical studies regarding the beneficial effects of TUDCA in delaying the progression of neurodegeneration, and may open doors for further clinical studies to be carried out, in different types of ND.

7. CONCLUSION AND PERSPECTIVES

The search for new therapeutic strategies has been emerging in the context of neurodegenerative diseases, since the population, life expectancy and socioeconomic burden have grown considerably in recent years. A crescent number of studies supports TUDCA as an important neuroprotective bile acid, as it improves many of the symptoms seen in several experimental models of neurodegeneration.

The mechanisms by which TUDCA acts are not fully understood, and should include several signaling pathways, such as those involving modulations in: a) ER stress, b) apoptosis, c) oxidative stress, and d) neuroinflammation (Figure 2). Importantly, most studies centered on understanding the molecular pathways activated by TUDCA were focused on apoptosis and related pathways. The large amount of available works showing the beneficial effects of this bile acid in neurodegeneration models, indicates TUDCA as a candidate with great potential in the treatment of these illnesses.

Further works are still needed to explore the intracellular pathways that mediate the therapeutic effects of TUDCA on neurodegeneration, as well as unraveling novel roles of TUDCA. The determination of the exact molecular pathways activated by TUDCA will be important for more clinical trials to be conducted in humans.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. LZ, LROR and HCLB contributed to the data collection and writing. JFV and EMC contributed to the discussion and writing. LZ contributed to the graphical designs. All authors reviewed and approved the final version of the manuscript.

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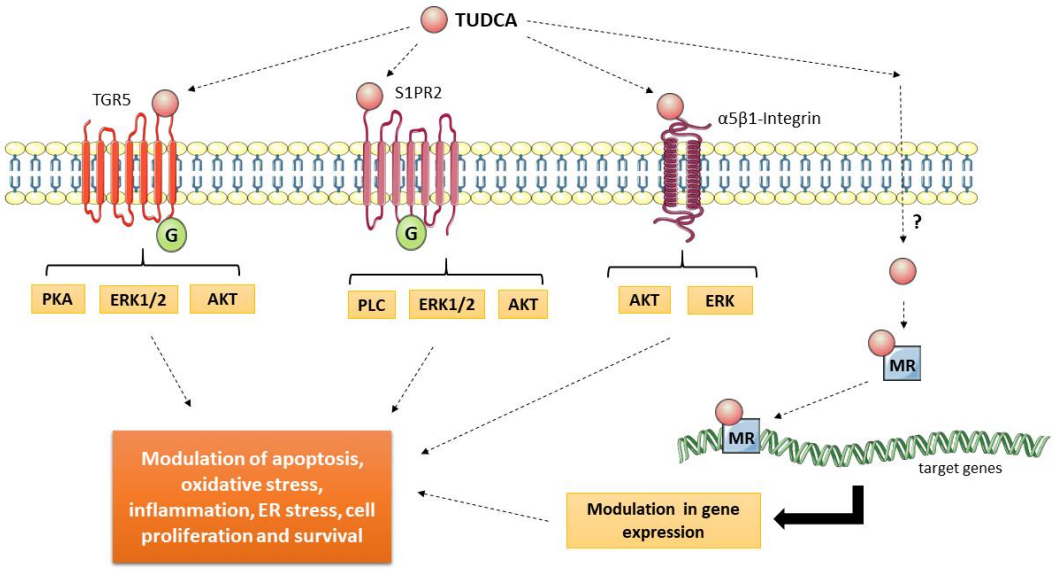
FIGURE LEGENDS

Figure 1. TUDCA and its receptors in the brain. Despite the large number of studies demonstrating the effects of the bile acid TUDCA in neurodegeneration, the TUDCA-activated receptor(s) in these models has not been fully elucidated yet. It is known that TUDCA has greater affinity with membrane receptors, such as TGR5, S1PR2 and α5β1 integrin. The activation of these receptors results in a complex intracellular signaling network, with the activation and inhibition of several molecular pathways. The specific pathways that are activated by TUDCA in a context of neurodegeneration remain unknown. However, the ERKs and AKT pathways call attention for being involved in several beneficial responses in the central nervous system, modulating apoptosis, ER stress, oxidative stress, neuroinflammation, cell proliferation and survival. Interestingly, it has been shown that TUDCA activates the cytosolic mineralocorticoid receptor in neurons, resulting in inhibition of apoptosis. However, the mechanisms involved in transporting this bile acid into neural cells are still poorly understood. It is known that in hepatocytes, TUDCA can be transported into the cell by NTCP channels and, thereby, activate intracellular receptors. Though, these channels are only found in hepatocytes. TUDCA: tauroursodeoxycholic acid; ERK: extracellular-signal-regulated kinase; PKA: protein kinase A; PLC: phospholipase C; MR: mineralocorticoid receptor; TGR5: Takeda G protein receptor 5; S1PR2: sphingosine-1-phosphate receptor-2; ER stress: endoplasmic reticulum stress.

Figure 2. TUDCA effects in experimental models of neurodegenerative disorders. The bile acid TUDCA has a wide range of actions in different cell types. The fact that this bile acid has an affinity for different receptors that are present in different tissues guarantees TUDCA to act in several biological processes. Briefly, in experimental models of AD, TUDCA prevents apoptosis from occurring, by attenuating several pro-

apoptotic pathways and stimulating anti-apoptotic mechanisms. TUDCA also reduces the amyloidogenic APP processing pathway and accumulation of amyloid β peptides in the hippocampus and frontal cortex, and decreases synaptic loss. All of these events contribute to the improvement in spatial, recognition and contextual memory in APP/PS1 double knockout mice. In experimental models of PD, TUDCA treatment prevents neuronal death, apoptosis and oxidative stress. TUDCA also reduces the generation of ROS, probably through the Nfr2 activation. Furthermore, TUDCA increases the expression of PINK1 and parkin, implying that TUDCA induces mitophagy. TUDCA also showed neuroprotective effects in a mice model of PD, by decreasing JNK activity, which exert a crucial role in dopaminergic neuronal death, reducing ROS production and neuroinflammation, and activating the pro-survival AKT pathway. Altogether, the effects culminated in the improvement of motor performance and symptoms typical of PD, such as spontaneous activity, ability to initiate movement and tremors. Ultimately, TUDCA prevents the striatal apoptosis and cerebral and striatal atrophy in 3-NP HD mice model, as well as reduces the accumulation of ubiquitin in the striatum of R6/2 transgenic mice, ameliorating their sensorimotor deficits. *In vitro* and *in vivo* data support that the effects of TUDCA mostly result in attenuating the processes of apoptosis, neuroinflammation and oxidative stress, culminating in the improvement of cognition and motor performance, affected by these diseases.


TUDCA
TGR5
S1PR2
α5β1-Integrin
G
G
?
PKA
ERK1/2
AKT
PLC
ERK1/2
AKT
AKT
ERK
MR
MR
target genes
Modulation of apoptosis, oxidative stress, inflammation, ER stress, cell proliferation and survival
Modulation in gene expression

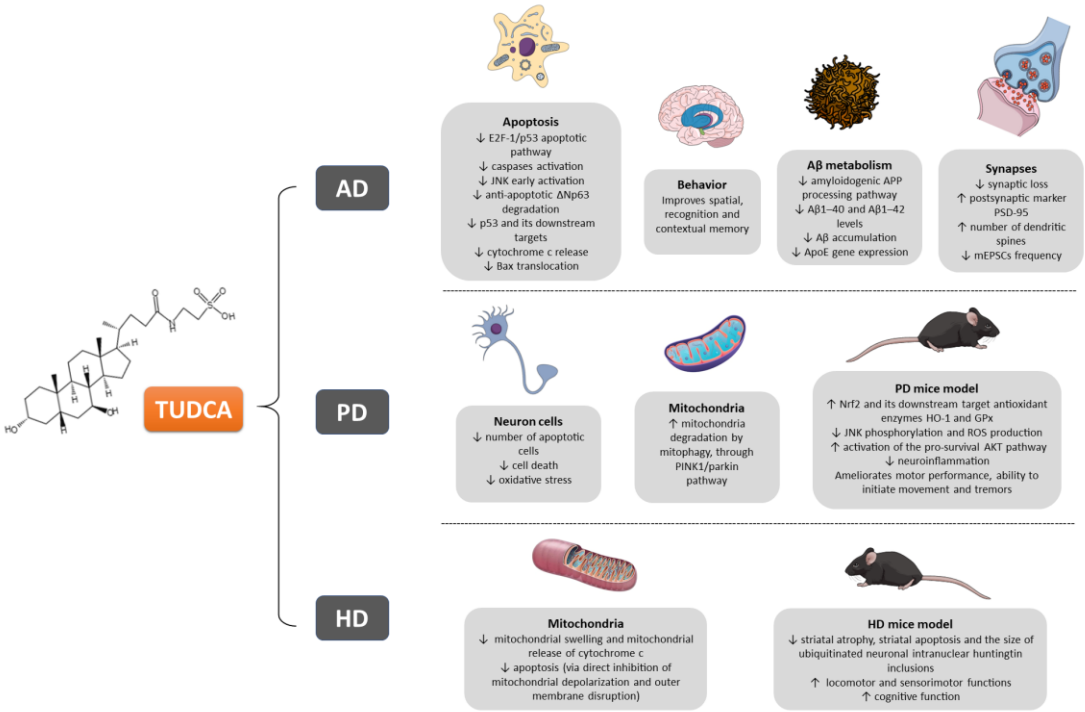

TUDCA
AD
PD
HD
Apoptosis
↓ E2F-1/p53 apoptotic pathway
↓ caspases activation
↓ JNK early activation
↓ anti-apoptotic ΔNp63 degradation
↓ p53 and its downstream targets
↓ cytochrome c release
↓ Bax translocation
Behavior
Improves spatial, recognition and contextual memory
Aβ metabolism
↓ amyloidogenic APP processing pathway
↓ Aβ1–40 and Aβ1–42 levels
↓ Aβ accumulation
↓ ApoE gene expression
Synapses
↓ synaptic loss
↑ postsynaptic marker PSD-95
↑ number of dendritic spines
↓ mEPSCs frequency
Neuron cells
↓ number of apoptotic cells
↓ cell death
↓ oxidative stress
Mitochondria
↑ mitochondria degradation by mitophagy, through PINK1/parkin pathway
PD mice model
↑ Nrf2 and its downstream target antioxidant enzymes HO-1 and GPx
↓ JNK phosphorylation and ROS production
↑ activation of the pro-survival AKT pathway
↓ neuroinflammation
Ameliorates motor performance, ability to initiate movement and tremors
Mitochondria
↓ mitochondrial swelling and mitochondrial release of cytochrome c
↓ apoptosis (via direct inhibition of mitochondrial depolarization and outer membrane disruption)
HD mice model
↓ striatal atrophy, striatal apoptosis and the size of ubiquitinated neuronal intranuclear huntingtin inclusions
↑ locomotor and sensorimotor functions
↑ cognitive function

Table 1: Ongoing Registered clinical trials with TUDCA and UDCA in neurodegeneration

Condition	Study Title	Clinical Trials Identifier:	Recruitment Status	Study Design and Interventions
Alzheimer's Disease	Study to Assess the Safety and Biological Activity of AMX0035 for the Treatment of Alzheimer's Disease (PEGASUS)	NCT03533257	Active, not recruiting	Drug: AMX0035 (combination therapy of TUDCA and Sodium Phenylbutyrate) Estimated Enrollment: 100 participants Allocation: Randomized Double-blind Phase: 2 Doses and Mode of administration: Not Available Duration: 6 Months Placebo-Controlled
Amyotrophic Lateral Sclerosis	Safety and Efficacy of TUDCA as add-on Treatment in Patients Affected by ALS (TUDCA-ALS)	NCT03800524	Recruiting	Drug: TUDCA Estimated Enrollment: 440 participants Allocation: Randomized Double-blind Phase: 3 Doses: 4 capsules (1 g), 250 mg capsules, twice daily 10-15 minutes after a meal Mode of administration: orally Duration: 18 months Placebo-Controlled
Progressive Multiple Sclerosis	A Trial of Bile Acid Supplementation in Patients With Multiple Sclerosis	NCT03423121	Recruiting	Drug: TUDCA Estimated Enrollment: 60 participants Allocation: Randomized Double-blind Phase: 1/2 Doses: 1 g of TUDCA twice daily in the form of four 250 mg capsules. Mode of administration: orally Duration: 16 weeks Placebo-Controlled
Amyotrophic Lateral Sclerosis	Open Label Extension Study of AMX0035 in Patients With ALS (CENTAUR-OLE)	NCT03488524	Enrolling by invitation	Drug: AMX0035 (combination therapy of TUDCA and Sodium Phenylbutyrate) Estimated Enrollment: 132 participants Allocation: N/A Open-label Phase: 2 Doses: twice daily- a combination therapeutic including 3 g of Phenylbutyrate and 1g TUDCA Mode of administration: orally Duration: 30 months
Parkinson's Disease	Brain Bioenergetics in Parkinson's Disease and Response to Repeated Oral UDCA Treatment	NCT02967250	Not yet recruiting	Drug: UDCA Estimated Enrollment: 20 participants Allocation: N/A Open-label Phase: 1 Doses: 50mg/kg/day (based on the use of 250 and 50 0mg capsules) of UDCA to be divided into 3 equal daily doses Mode of administration: orally Duration: 6 weeks
Parkinson's Disease	Trial of Ursodeoxycholic Acid (UDCA) for Parkinson's Disease: The "UP" Study	NCT03840005	Recruiting	Drug: UDCA Estimated Enrollment: 30 participants Allocation: Randomized Double-blind Phase: 2 Doses: 30 mg/kg daily Mode of administration: orally Duration: 48 weeks Placebo-Controlled
Huntington's Disease	Ursodiol in Huntington's Disease (UDCA-HD)	NCT00514774	Unknown	Drug: UDCA Estimated Enrollment: 21 participants Allocation: Randomized Double-blind Phase: 1 Doses: 300 or 600 mg twice daily Mode of administration: orally Duration: 28 days Placebo-Controlled

TUDCA: Tauroursodeoxycholic acid, UDCA: Ursodeoxycholic acid