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# The therapeutic potential of chemical chaperones in protein folding diseases

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**Abbreviations:** PQC, protein quality control; UPR, unfolded protein response; Hsps, heat shock proteins; TMAO, trimethylamine N-oxide; PrPC, cellular form of prion protein; PrPSc, disease-associated prion protein; DMSO, dimethyl sulfoxide; PBA, 4-phenylbutyrate; CJD, Creutzfeldt Jakob disease; ALS, amylotrophic lateral sclerosis; HD, Huntington disease; AD, Alzheimer's disease; PD, Parkinson disease; HDAC, histone deacetylase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; BA, bile acid, DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; TTR, transthyretin

Several neurodegenerative diseases are caused by defects in protein folding, including Alzheimer, Parkinson, Huntington, and prion diseases. Once a disease-specific protein misfolds, it can then form toxic aggregates which accumulate in the brain, leading to neuronal dysfunction, cell death, and clinical symptoms. Although significant advances have been made toward understanding the mechanisms of protein aggregation, there are no curative treatments for any of these diseases. Since protein misfolding and the accumulation of aggregates are the most upstream events in the pathological cascade, rescuing or stabilizing the native conformations of proteins is an obvious therapeutic strategy. In recent years, small molecules known as chaperones have been shown to be effective in reducing levels of misfolded proteins, thus minimizing the accumulation of aggregates and their downstream pathological consequences. Chaperones are classified as molecular, pharmacological, or chemical. In this mini-review we summarize the modes of action of different chemical chaperones and discuss evidence for their efficacy in the treatment of protein folding diseases in vitro and in vivo.

## Introduction

The misfolding of proteins is a common event in cells. This can be caused by chance, by environmental factors or by mutations that make the native protein conformation less stable. When proteins misfold, they expose hydrophobic segments that are normally buried in the core of their native conformation. This exposure promotes the formation of intermolecular binding and subsequent aggregation. It is generally accepted that these aggregates are toxic and their accumulation is the cause of neurodegenerative disorders like Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), and prion disease.[1]

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To avoid accumulation of these neurotoxic species, cells have evolved a protein quality control (PQC) system which supervises protein folding and eliminates misfolded proteins before they can exert toxic effects.[2] Unfortunately, as we age, the delicate balance of the synthesis, folding, and degradation of proteins can be altered and the load of misfolded protein may overwhelm the PQC system allowing the accumulation of toxic protein aggregates. Under this cellular stress, the unfolded protein response (UPR) is activated.[2] Through this response, the cell tries to restore its normal function by stopping protein synthesis and increasing the production of chaperones involved in protein folding. If this response is not sufficient to stop the accumulation of unfolded protein, the UPR directs the cell toward apoptosis. Improving the efficiency of the PQC system, therefore, is one approach to combating protein folding diseases.

Chaperones are one of the major players of the PQC system. As traditionally defined, they oversee the correct folding and assemble of proteins, thus preventing their degradation or aggregation and ensuring their appropriate trafficking and function. The overall proteostatic function of chaperones makes them prime candidates for therapeutic agents for neurodegenerative disease. They are classified into 3 groups: molecular, pharmacological, and chemical.

Molecular chaperones are proteins that interact with the nonnative state of other proteins to assist them in their folding or unfolding and their assembly or disassembly. They are not present in the final functional protein structure. They represent the first and most potent line of defense against protein misfolding and the aggregation process.[3] For example, overexpression of heat shock proteins (Hsps), the major molecular chaperones in cells, has been shown to be neuroprotective in neurodegenerative diseases,[4] and this has led to studies of Hsps as potential therapies.[5]

Pharmacological chaperones are low molecular weight compounds which specifically bind proteins and induce refolding or structure stabilization, restoring protein function.[6] They can be enzyme or receptor ligands or molecules which selectively bind to a particular native conformation of a protein to increase its stability.

Chemical chaperones can be divided into 2 groups: osmolytes and hydrophobic compounds.[7] They are also low molecular weight compounds but they have a nonspecific mode of action and in some cases cannot bind directly to the proteins. Unlike the pharmacological chaperones, these molecules usually only have effect at high concentrations (molar), so they have been largely neglected as therapeutic agents. However, recently some of these molecules are receiving increasing attention as potential treatments for neurodegenerative conditions given their complex mechanisms of action which likely act at different levels of the neuropathology cascade.

In this mini-review we will focus on the role and therapeutic potential of chemical chaperones in protein folding diseases, including prion disease.

## Osmolyte Chaperones

Cellular osmolytes are ancient members of stress responses. They play an important role for organisms exposed to stress conditions such as fluctuating salinity, desiccation, or extreme temperatures.[8] The major osmolytes in eukaryotes are restricted to a few classes of low molecular weight compounds: free amino acids and amino acid derivatives (e.g., glycine, taurine, β-alanine), polyols (e.g., glycerol, sucrose), and methyl-amines (e.g., trimethylamine N-oxide [TMAO]).

**Mechanism of action**

Under denaturing environmental stresses, the intracellular milieu is enriched with organic osmolytes and these osmolytes increase the stability of proteins without affecting their activity.[8] Particular osmolytes appear to be involved in particular stress conditions. For example, polyols protect cells against extreme temperature and dehydration, amino acids protect against extracellular environments that are high in salt concentration, and methylamines are present in urea-rich cells, protecting against the deleterious effect of urea on protein structure.[8]

Despite these differences, osmolytes share the same nonselective mechanism for stabilization of protein structure; they alter solvent properties. A peptide backbone is hydrophilic but amino acid side chains vary in their hydrophobicity. Thus there is a balance of backbone and side chain interaction in a given solvent that determines the free energy of a folded or unfolded state. Osmolytes sequester water molecules, leaving a hydrophobic environment around the protein. This increases the free energy of the protein's unfolded state more than its folded state, thereby shifting the folding–unfolding equilibrium toward the folded state, where the hydrophilic protein backbone minimizes its exposure to the hydrophobic surroundings.[9-11]

In addition to their direct effect on the protein conformation and folding, osmolytes modulate the function of molecular chaperones,[12,13] further improving the efficiency of the PQC system.

**Evidence for therapeutic effects in vitro**

The effect of osmolyte chaperones on neurodegenerative-related protein misfolding has been well studied in vitro.

Experiments with α-synuclein, the protein associated with PD, demonstrated that the methylamine TMAO induced conversion of the unfolded protein into its native state.[14] Polyols and TMAO also modulated the aggregation properties of Aβ peptide, which accumulates in AD, accelerating the conformational change from random coil to β-sheet.[15,16]

Within prion diseases, TMAO and glycerol prevented conversion of the cellular form of prion protein ($PrP^{C}$) into its infective form ($PrP^{Sc}$) in scrapie-infected mouse neuroblastoma cells, without affecting the existing population of $PrP^{Sc}$.[17] In a PrP H187R (PrP187R) cell model, which is a prototype of familial Creutzfeldt Jakob disease (CJD), glycerol also reduced the lysosomal accumulation of PrP187R and facilitated its transport to the cell surface.[18] Molecular dynamics simulations studying the conversion of PrP at low pH revealed that TMAO prevented residues that are key to conversion from assuming an extended sheet structure.[19]

**Evidence for therapeutic effects in vivo**

Oral administration of 2% of trehalose solution (starting at 21 d of age and continuing until the day the mice were killed) has been shown to improve motor dysfunction and extend lifespan in a transgenic mouse model of HD by minimizing the aggregation propensity of the disease-associated polyglutamine-containing protein Huntingtin.[20]

Although not a naturally occurring chaperone, the well-studied osmolyte DMSO significantly prolonged disease incubation time and delayed the accumulation of $PrP^{Sc}$ in hamsters intracranially inoculated with prions. This was achieved with oral administration of a 7.5% solution (starting at day 0 or 14 dpi). It should be noted however, that clear adverse effects such as weight loss were observed.[21]

**Future therapeutic potential**

Despite their high efficiency as anti-aggregation agents and beneficial effects in animal models of neurodegenerative diseases, very few of these osmolytic chaperones have entered into clinical trials because of issues related to their toxicity (the majority of these chemical chaperones require high concentrations for effectiveness) and their lack of specificity. Instead, the group of hydrophobic chaperones, which are much less toxic, are being increasingly examined as therapeutic agents.

## Hydrophobic Chaperones

Several molecules have been classified as hydrophobic chaperones, including sodium 4-phenylbutyrate (PBA) and bile acids. The general mechanism of action proposed for chemical chaperones involves the interaction of hydrophobic regions of the chaperone with exposed hydrophobic segments of the unfolded protein. This interaction protects the protein from aggregation. However, whether PBA and bile acids truly demonstrate this behavior is unclear. They do reduce aggregate accumulation in vivo and in vitro and revert endoplasmic reticulum (ER) stress, but it has been recently demonstrated that these molecules have

more complex mechanisms of action which influence many levels of regulation.

**4-phenylbutyrate**

Sodium 4-phenylbutyrate (PBA) is an orally bioavailable, blood brain barrier (BBB) permeable, short-chain fatty acid that has been approved by the Food and Drug Administration (FDA) for treatment of urea cycle disorders. PBA has potential benefit for a wide variety of diseases like cancer, cystic fibrosis, thalassemia, spinal muscular atrophy as well as protein folding diseases such as type 2 diabetes mellitus, amyotrophic lateral sclerosis (ALS), HD, AD, and PD.[22-30]

Although PBA has been classically described as a chemical chaperone, based on its effects on ER stress and aggregate accumulation, the actual molecular mechanisms involved in its beneficial effects are not completely clear.

*Mechanisms of action*

Two primary mechanisms of action have been proposed for PBA: chemical chaperone and histone deacetylase (HDAC) inhibitor. Modifications to PBA structure that remove HDAC inhibitory activity do not abolish its effects on protein aggregation or ER stress, suggesting that the protective effects are not based on HDAC inhibition alone but rather may involve direct chaperone-like interactions.[31] At the same time, the HDAC inhibitory property of PBA allows it to regulate the transcription of many genes involved in the UPR system; PBA induces the synthesis of molecular chaperones[32] while downregulating general protein synthesis.[33] Also of note, epigenetic regulators related to histone acetylation are critical regulators of neuronal gene expression. It has been recently suggested that HDAC inhibitory activity may be related to the enhancement of synaptic plasticity, learning, and memory, indicating that compounds like PBA might be a useful class of therapeutic agents for neurodegenerative disorders.[34] Indeed, PBA has been studied as a therapeutic agent in vitro and in vivo.

*Evidence for therapeutic effects in vitro*

PBA can inhibit the aggregation of recombinant α-synuclein aggregation in vitro, likely through its chaperone effect on hydrophobic interaction.[35] PBA also has neuroprotective effects in cell culture studies, likely mediated through HDAC inhibition. In neuronal cell culture models of AD, treatment with PBA led to neuroprotection associated with a decrease in tau phosphorylation. It also modulated the processing of the amyloid precursor protein (APP).[36,37] In a cell model of PD, PBA increased the expression of genes important for moderating oxidative stress and protein aggregation, thereby reverting the pathology.[28]

*Evidence for therapeutic effects in vivo*

There have been encouraging results from PBA treatment of several animal models of neurodegenerative disease.

Oral administration of 0.5% PBA solution to AD mouse models significantly reduced the number of plaques in the hippocampus and reversed cognitive deficits.[38,39] It has been suggested that at least part of this neuroprotective effect is due to the chaperone-like activity of PBA.[36]

Neuroprotective effects of PBA were also assessed on rotenone-induced PD mouse models. Protection against neurodegeneration was observed when PBA at a dose of 120 mg/kg was intraperitoneally injected 30 min before each oral administration of rotenone.[35] Additionally, oral administration of PBA (200 mg/kg/day 3h after injection with MPTP) in an acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD inhibited the expression of proinflammatory molecules and attenuated the production of reactive oxygen species from activated microglia.[40]

In HD mouse models, daily intraperitoneal injections of PBA (100 mg/kg/day) ameliorated the neurodegenerative phenotype, significantly extending survival and attenuating both gross brain and neuronal atrophy.[27,41] Through its HDAC inhibitory activity, PBA increased mRNA for components of the ubiquitin-proteosomal pathway and downregulated caspases implicated in apoptotic cell death. Of note, suberoylanilide hydroxamic acid, another HDAC inhibitor, has been also able to ameliorate motor deðcits in a mouse model of HD.[42]

*Future therapeutic potential*

While PBA can be utilized with a good efficiency by oral supplementation and treatment seems to have no severe side effects, the main drawback to its therapeutic use is the high dosage required. If a direct translation of the effective dosage in AD mice (200 mg/kg) is done, up to 15 g/day would be required for AD treatment in humans. This concentration was the maximum tolerated in a human tolerability study of HD patients.[43] It is possible that modifications of this molecule can be made in order to improve its efficiency. In one study, 4-PBA derivatives carrying a *p*-nitro, *p*-amino, or *p*-methoxy group on the benzene ring were able to influence anti-aggregation activity, although the effects were mild.[31]

**Lipids and detergents (bile acids)**

Bile acids (BAs) are acidic steroids that are synthesized from cholesterol in the liver. They are secreted into the intestine where they can be dehydroxylated by bacteria to become secondary BAs like deoxycholic acid (DCA) or ursodeoxycholic acid (UDCA). Secondary BAs can then be returned to the liver and conjugated with amino acids to generate conjugated BAs such as tauroursodeoxycholic acid (TUDCA) which is UDCA conjugated with the amino acid taurine. BAs are the major constituents of bile. While their main role is the solubilization of dietary fats and fat-soluble vitamins to improve absorption from the intestinal lumen, in recent years, neuroprotective functions have been attributed to BAs.[44,45]

*Mechanisms of action*

TUDCA and UDCA can reduce the accumulation of toxic aggregates in different experimental models of neurodegenerative diseases. As such, they have been classified as chemical chaperones. However, as is the case for PBA, the observed treatment benefits may also be the result of other functions attributed to these bile acids.[46]

The cytoprotective effects of UDCA and TUDCA have been attributed to the reduction of reactive oxygen species formation,[47] the prevention of mitochondrial dysfunction,[48] and the inhibition of apoptosis through both the intrinsic[49] and the extrinsic pathway.[50]

These steroids can also activate specific nuclear receptors and G protein–coupled receptors influencing the expression of genes

that encode proteins involved in the regulation of glucose, fatty acid, lipoprotein synthesis, energy metabolism, and the regulation of their own synthesis.[51]

*In vitro data*

There is in vitro evidence to support a chaperone activity for BAs. Studies of millimolar concentrations of TUDCA and UDCA have demonstrated their inhibitory effects on the thermal aggregation of different proteins.[52,53] This effect may have been secondary to the direct stabilization of the protein in question or through direct interaction with other molecular chaperones, enhancing their function.[52,54] However, this chaperone effect is not observed for all proteins; when neurodegenerative disease-related proteins like Aβ peptide were tested, TUDCA was found ineffective at preventing aggregation in vitro,[55] although these latter experiments were performed with lower concentrations of TUDCA (micromolar). Also of note, the cytotoxic steroid DCA actually enhanced the in vitro aggregation of prion protein (PrP).[56]

Despite the lack of effect on aggregation of Aβ peptide in vitro, TUDCA strongly inhibited apoptosis in a cell culture model of AD in which cells expressed the Dutch mutation (E22Q) in amyloid precursor protein.[55] Thus the ability of BAs to directly interact with and influence protein misfolding could be protein specific and they may exert protective effects via other mechanisms when used in cellular environments.

*In vivo data*

The neuroprotective action of BAs has been tested in several animal models of neurodegenerative diseases. In transgenic mouse models of Familial Amyloidotic Polyneuropathy, TUDCA significantly reduced transthyretin (TTR) toxic aggregates without affecting TTR aggregation in vitro,[57] again suggesting that this effect was not due to a direct stabilization of the native structure of the protein but rather an effect on other cellular processes such as apoptotic or oxidative mechanisms involved in the early stages of pathology. Feeding APP/PS1 transgenic mice with a diet supplemented with TUDCA reduced the accumulation of Aβ deposits and ameliorated memory deficits. This effect was associated with but not limited to a reduction of γ-secretase activity.[58] Administration of TUDCA led to a significant reduction in neuropathology and improved the locomotor and sensorimotor deficits of a transgenic HD mouse model.[44] Additionally, intraperitoneal injection of TUDCA in mouse model of PD prevented dopaminergic cell death through the activation of pro-survival pathways.[59]

*Future therapeutic potential*

Bile acids, in particular TUDCA and UDCA, have received increasing attention as potential treatments for neurodegenerative conditions given their anti-amyloidogenic activity and their ability to modulate apoptotic pathways.[60] TUDCA and UDCA have therapeutic advantages over other compounds with chaperone activity. These BAs are orally bioavailable, BBB permeable, and have a low toxicity profile. In addition, TUDCA has been FDA-approved for use in humans to treat primary biliary cirrhosis.

## Translation to Clinical Trials

There have been many efforts to develop small molecules that can inhibit the aggregation of proteins. Numerous anti-amyloidogenic compounds have been discovered either by screening large libraries of organic molecules or by rationally designing compounds based on the structure and dynamics of the protein involved.[61,62] The use of these molecules can partially overcome and in some cases complete reverse the pathology in vitro and in vivo using models of neurodegenerative diseases. Unfortunately, those compounds which have been tested in clinical trials have had discouraging results to date.[63]

There are several factors that could explain why these compounds have failed in humans so far. A major problem is that no effective preclinical diagnoses are readily available for these diseases. It may be that once clinical symptoms appear, the level of protein aggregation and accumulation is too high to be affected by prevention of further misfolding. Compounds designed to act exclusively at the initiation of aggregation will be effective only if given very early in disease. Chaperones, as protein stabilizing molecules, may have less effect on pre-formed aggregates than on the monomer proteins themselves. Designing chaperones that more directly affect the aggregates is challenging because the structure of the toxic aggregates and their mechanism(s) of conversion remain unclear. As newer research begins to reveal more of this structural information, there may be more success in inhibitory molecule design. For example, the extent of β sheet content within a toxic oligomeric species may direct the planar attributes of the inhibitory molecule.[64] Finally, if an effective anti-aggregation molecule is discovered or successfully synthesized, this drug must cross the BBB and cannot be toxic at the effective concentration.

The most effective treatment regimen may involve a combination of drugs, some which stop protein accumulation, some which promote clearance of toxic aggregates, and others which act downstream to offset the neurodegenerative cascade. In this context, compounds which themselves have a multi-modal/multi-target mechanism would be ideal. TUDCA, UDCA, and PBA may fulfill this role, in that they are not only able to counterattack the misfolding step but also modulate apoptosis, gene expression, and probably other biological processes involved in the neuropathogenesis. These natural compounds also have the advantage of being orally bioavailable, being BBB permeable, having a low toxicity profile, and already being FDA-approved drugs. Clinical trials are currently underway to study the tolerability and efficacy of TUDCA and UDCA in patients with ALS, TTR amyloidosis, and HD.

## Conclusion

As our life expectancy and population numbers continue to increase, so will the prevalence and socioeconomic burden of neurodegenerative diseases. Given this, it is of some urgency to

find viable therapeutic strategies. Since the accumulation of misfolded host proteins in the brain is the putative cause for many of these diseases, stabilizing native protein structure is a logical approach. TUDCA and UDCA, which can have anti-aggregation effects as well as effects on apoptosis regulation, may represent safe and effective compounds which act at more than one level of the neuropathogenic cascade. More studies with these promising compounds should be pursued to fully understand their mechanism(s) of action and to expand the repertoire of treatable neurodegenerative diseases.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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