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Activation of Heat Shock Proteins by Nanocurcumin to Prevent Neurodegenerative Diseases

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Abstract

Protein misfolding and its progressive accumulation in central nervous system are the common features and the leading cause of several neurodegenerative diseases. Currently no treatment is available to complete removal of these aggregates and rescue neuronal death, synaptic impairment as well as cognitive deficits. Interesting, the cell itself has a fantastic defense mechanism to remove these aggregates. One such crucial mechanism is molecular chaperones such as heat shock proteins. In general, the larger protein aggregates are removed by this system, and degraded through the proteasome or autophagy pathway. Dysfunction or dysregulation of heat shock system has been observed in several neurological diseases, which severely interfere cellular protein clearance mechanism. Therefore, maintenance or balancing of these endogenous protein clearance pathways is a promising approach to remove these protein aggregates. Several small molecules, drugs, and phytochemicals have been studied to promote the activation of this system. Recently, as a potent anti-amyloid polyphenol, curcumin has drawn special attention for the treatment of several brain disorders including several neurodegenerative diseases. It is effective to boost cellular protein clearance system; therefore, it is considered one of the most promising compounds to rectify dysfunction of heat shock system in these disorders. Whereas, because of its low water solubility and fast degradation, the bioavailability of curcumin is very poor. Using nanotechnology, recently several research groups formulated "nanocurcumin" to improve its bioavailability and significantly increases it's therapeutic efficacy against cancer, but scanty of data are available about it's role on boosting heat shock system to prevent protein misfolding and neurodegeneration. In this review we will discuss the current knowledge about the importance of nanocurcumin and its pivotal role on activation of heat shock system to combat against neurodegenerative diseases caused by protein misfolding.

Keywords: Protein misfolding; Neurodegenerative diseases; Molecular chaperones; Heat shock system; Curcumin; Nanocurcumin.

Abbreviations

HSP: Heat Shock Protein, HSS: Heat Shock System; HTT: Huntingtin, Rnai-Interference Ribonucleic Acid; FDA: Food And Drug Administration; ER: Endoplasmic Reticulum; aβ: Amyloid Beta Protein; PRPsc: Prion Protein Scrapie Form; PRPc: Prion Protein Common/Cellular; AD: Alzheimer's Disease, PD: Parkinson's Disease; HD: Huntington's Disease, MS: Multiple Sclerosis; Polyq: Poly Glutamine; AIF: Apoptosis Inducing Factor; CMA: Chaperone Mediated Autophagy; TG: Transgenic; KI: Knock In; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; EC: Effective Concentration; PIB: Pittsburg Compound; FDG: Fluorodeoxyglucose; PET: Positron Emission Tomography; BACE: Beta Secretase; PS1: Presenilin-1; NFTS: Neurofibrillary Tangles; PPM: Parts Per Million; APP: Amyloid Precursor Protein; CHO: Chinese Hamster Ovary; CAG140ki: Cag140 Knock In; TG: Transgenic; APPsw: Amyloid Precursor Protein Swedish Mutation; HTAUTG: Human Tau Transgenic.

Introduction

Abnormal protein aggregation and their dysfunction is the leading cause of neuronal death observed in several neurodegenerative diseases [1-3]. It comprises more than fifty different medically challenged metabolic, neurodevelopmental or neurodegenerative disorders, for which still there is no cure [4]. Most of these diseases are silent killer, progress slowly and persist rest of the life [1]. The cardinal features of most of these devastating diseases are synaptic loss/failure, neurobehavioral abnormalities and executive dysfunctions including impairment of learning and memory. Accumulated knowledge supports the idea that those diseases might start at early stage of life, but manifestations may come at later stage especially during aging [1,5]. The most common neurodegenerative disease is Alzheimer disease (AD), where amyloid beta protein (Aβ) is deposited as senile plaque mainly in extracellular spaces, whereas microtubule stabilizing protein tau is deposited as neurofibrillary tangle (NFT) in intracellular spaces [1,6]. Similarly, α-synuclein, huntingtin (HTT), prion are deposited in Parkinson's, (PD) Huntington's (HD) and prion diseases respectively [1-6] (Table 1). However, in most of these disorders all these amyloid proteins undergo conformational changes and become misfolded under certain conditions and deposited as insoluble proteinaceous aggregates inside or outside of cells leads to degenerative changes [1,2,6]. Therefore, adequate treatment to be started before onset of these diseases in order to prevent later

downstream toxicity of those protein aggregates. Several investigators have attempted to clear these protein aggregates from cells, but we yet have not achieved with significant solution. Recently, induction of the endogenous protein clearance pathways such as activation or expression of heat shock proteins (HSPs) draw a special attention as therapeutic strategies to remove those abnormal protein aggregates [7].

The heat shock system (HSS) is a highly conserved cellular defence mechanism, which regulates various cellular functions including protein folding, refolding of partially denatured proteins, protein transport across membranes, cytoskeletal organization, degradation of disabled proteins, and apoptosis [8-10]. It also acts as cytoprotective factor against deleterious environmental stresses, and also activates a wide range of client substrates, including kinases, hormone receptors, and transcription factors as well as oncogenic proteins [10,11]. Different HSPs are found to be localized in synapses and axons and misregulated in different brain diseases. They are implicated in regulating disposal of toxic aggregation such as amyloid plaques and NFT in AD [12,13], α- synuclein in PD [14,15], huntingtin (HTT) in HD [12,16-18] and prion protein in Creutzfeldt-Jakob Disease (CJD) or prion diseases [19,20] via multiple mechanisms [21-23]. Several species of HSPs can bind in vitro and/or in vivo directly to tau, independent of tau phosphorylation, facilitating microtubule polymerization, and limiting tau aggregation [24-26]. Overexpression of certain HSPs decrease the amount of insoluble Aβ, tau, reduced tau phosphorylation and increased tau stability, promote tau binding to microtubules, and decrease the toxicity in vitro and in vivo [25]. In contrast, down regulation of HSPs by RNA-mediated interference (RNAi) had the opposite effects [12]. Most importantly, HSPs can also bind to mutant HTT [17,27], α-synuclein or prion oligomers or prefibrillar structures, thus interfere in formation of their low molecular weight soluble oligomers or higher order insoluble structures respectively [28,29]. They also play pivotal role in regulation of ubiquitin proteasome and the autophagic-lysosomal pathways for their precise functions [28-30].

Knowing the broad cytoprotective properties of HSPs, it is important to find out any molecule/drug, which is safe and capable of inducing the heat shock response and prevent neurodegeneration. Curcumin is one such safe and United States Food and Drug Administration (FDA) approved naturally occurring amyloid binding polyphenolic compound. The pleotropic actions of curcumin including anti-amyloidogenic, neuroprotective [31] antioxidant, anti-inflammatory, and anti-proliferation [32,33] suggesting that curcumin might be one of the most propitious compound for treatment of brain diseases. Whereas in case of neurodegenerative diseases, the potential roles of curcumin as a drug target are not fully understood [34]. It may modulate different regulatory pathways, signal transduction etc [32]. Recently, several research groups found strong link between curcumin and its positive regulation of HSPs in different neurological diseases [13,31,35]. Unfortunately, low solubility and less bioavailability is an important issue for therapeutic use of curcumin. To resolve this problem, several research groups formulated nanocurcumin by mixing curcumin with different combination of lipid nanoparticles [36-38]. In this review, we will discuss the significant details of advantages of nanocurcumin formulation, and its ameliorative effect on common dysfunction of endogenous protein clearance pathways such as heat shock responses to prevent neurodegenerative diseases.

Major Protein-Misfolding Diseases

Protein misfolding diseases or proteopathies area the class of diseases, which results loss or degeneration and dysfunction of cells, tissues, organs due to accumulation of functionally inactive proteins [1-6]. More than fifty different protein-misfolding diseases have been well characterized and below table we have mentioned very basic information about only four major neurodegenerative diseases (Table1).

Diseases involved	Genes involved	Risk Factors	Proteins	Pathology	Affected Brain Areas	Symptoms
Alzheimer's tangle	APP Presnilin-1,2	Apo E4	Aß,Tau	Aß-plaque, Tau	Hippocampus, frontal cortex	Memory loss Personality change, worried, depressed
Parkinsons Parkin UCHL-1,LRRK-2	α-synuclein	Tau linkage tau	α-synuclein	Lewy body tangle	Substantia nigra,Striatum PFC	Impairment of sensory motor coordination, cognition
Huntington	Huntington	No of CAG repeats in HTT allele	Huntington in cytoplasm and nucleas	Inclusion bodies	StriatumUncontrolled clumsiness ,Balane impairment	Uncontrolled clumsiness, Balane impairment
Prion	PRNP	Homozygosity at Prion codon 129	Pr Psc	Prion plaque	Whole CNS	Memory loss, Personality change, movement disorder

Table 1: Showed four major protein misfolding neurodegenerative diseases, gene involved, risk factors, protein involved, neuropathological signs, affected brain areas, and their symptoms [1-6].

Heat shock system: In general, most eukaryotic cells constitutively express a group of proteins, which have pivotal role in rectifying misfolded proteins known as HSPs [39,40]. In healthy organisms, HSPs maintain protein quality control and targeting abnormal or inactive proteins for their degradation [8,25,26,41,42]. Their expression markedly enhanced under extreme heat stimulus and also under certain stress environment [43,44]. Expression of several HSPs have been observed in different cellular stimuli and according to the

approximate molecular size or function, the principal HSPs are categorized into six conserved classes: the small HSPs (15 to 30 kDa, HSP10, HSP26/27) and larger HSPs such as HSP40, HSP60, HSP70, HSP90, HSP100/104/110 [43] (Table 2). They are mainly localized in cytoplasm, and also in cellular organelles such as mitochondria, endoplasmic reticulum (ER) and sometimes in the nucleus [45]. Under normal condition, the levels of HSPs are tightly regulated or maintained by local cellular environment, but when the load of misfolded protein aggregates cross the limit, tremendous deterioration of HSPs defense system has been observed and eventually they fail to respond. To cope up with this stress condition, induction of HSS has been noted.

HSPs	MW(kDa)	Localization	Co-localization	Functions	Involved in diseases
HSP10, ER,	10	Mitochondria, cytosol, nucleus	Aβ	Protein folding	AD, MS, tauopathies
HSP27	20-30	Cytosol, ER, nucleus, HTT, α-synuclein ,, Cytosol	Tau, Aβ, HTT, α-synuclein, Protein folding	Protein degradation, HD, PD	AD, HD, PD HSP40
HSP60	60	Mitochondria	Aβ	Prevent protein aggregation	AD
HSP70	70	Cytosol, ER, nucleus,, mitochondria	Aβ, HTT, α-synuclein, PrPc	Protein folding/unfolding	AD, HD, PD, prion, MS
HSP90	90	Cytosol, ER, as transcription factor	Aβ, HTT, α-synuclein, PrPc	Protein degradation & acts	AD, PD, HD
HSP104/110	100-110	Cytosol, ER	α-synuclein, PrPc	Thermal tolerance	

Table 2: Different species of HSPs, their cellular localization and involvement in different neurodegenerative diseases.

In contrast, overproduction of certain HSPs can lead to development of disease including cancer [44]. In addition, they also regulate the ubiquitin proteasome system or the autophagy pathway, hence HSPs play pivotal role in elimination of the most toxic aggregates, the soluble oligomers [25,46]. The removal or degradation of cellular debris by HSPs depends on type of proteins, the aggregate size, and their nature of misfolding.

Role of Specific Hsp in Different Neurodegenerative Diseases

Recent research revealed that soluble oligomers are the main culprit for neuronal death and HSPs can interacts with the most toxic oligomeric precursors and perhaps also with misfolded conformations of monomers [1-6]. Because of their role in protein folding and maturation as well as the renaturation of misfolded proteins, since last few years induction of HSPs has emerged as a potential targets for the treatment of many protein misfolding diseases including neurodegenerative diseases [7,14,44,47]. After synthesis they can be transported to synapses and axons and binds to aggregation or misfolded proteins. Experimental data evidenced that the early synaptic and axonal abnormalities in AD, PD and HD may be reversed by HSPs, (Figure 1), whereas in severe cases or advance stages, it is beyond their control [12,25,27]. In case of AD, it is assumed that HSPs may reduce Aβ aggregation by interfering with the amyloid precursor protein (APP) secretory pathway [48]. Among all these HSPs, HSP70 and HSP90 have most prominent in protein degradation [13,21]. Both HSP70 and HSP90 can promote tau solubility and tau binding to microtubules as well as reduce insoluble tau and tau phosphorylation [13,21]. Whereas, levels of HSP90 are inversely associated with granular tau oligomers and neurofibrillary tangles (NFTs) in AD [49] and in a mutant tau model [21]. Overexpression of inducible HSP70 reduces soluble and insoluble tau levels in 30- month-old mice [50]. Similarly, HSP70 has been shown to bind to HTT Exon 1 containing a poly glutamine (polyQ) expansion (a hallmark neuropathology observed in HD) in vitro and in yeast [16] and in mammalian cells [17]. It is co-localized with polyQ aggregates in vitro and in vivo, thus HSP70 might prevent aggregation by binding to a polyQ protein [12,17]. HSP70 also can inhibit oligomerization and fibril formation and makes Aβ, α-synuclein, prion, polyQ aggregate more soluble [12]. Recent experimental reports suggested that HSP40 and HSP70 both inhibit amyloid toxicity in cellular models and in vivo. Both of them can inhibit caspase-3 and caspase-9 activity, inhibit apoptotic-inducing factor (AIF) in HTT-transfected cells, indicating that they are directly involved in apoptosis [16,51,52]. Further, tau, APP and HTT are caspase substrates and HSPs are known to prevent caspase activation [10,51]. Similarly, HSP90 and its dependent client proteins are crucial for refolding denatured or misfolded tau, Aβ, α-synuclein, HTT, prion as well as the conformational maturation of several other nascent polypeptides into their biologically active structures. Not only those, HSPs are also associated with signalling cascades in normal cells [25,30]. Whereas, heat shock cognate 70 (HSC70: a constitutive expressed molecular chaperone) have important role during chaperone-mediated autophagy (CMA) [29,53,54], a special cellular clearance mechanism, which can remove larger amyloid, aggregates. Moreover, HSPs and their co-chaperones direct the misfolded/aggregated proteins to the degradation machineries such as proteasome system for their efficient degradation [7].

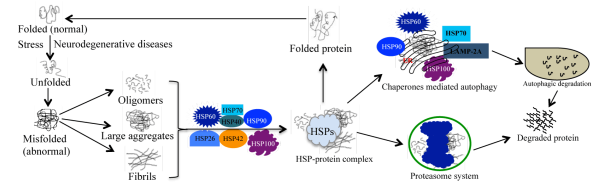

Figure 1: Experimental data evidenced that the early synaptic and axonal abnormalities in AD, PD and HD

Natural Polyphenols to Activate Heat Shock Response

There are several interests to investigate natural polyphenols as HSP inducer to prevent neurodegeneration. They are basically nontoxic, safe, easily available, low cost and can be administered orally. Several phenolic compounds derived from traditional medicinal plants have antioxidant, anti-inflammatory properties, but only few of them are potent HSPs activators/modulators. Among them mostly studied is geldanamycin (an antibiotic originated from *Streptomyces hygroscopicus*) as HSP90 inhibitor [55]. Recently, several other bioactive compounds like curcumin [13], celastrol [56], gambogic acid [57] and withaferin-A [58] have been identified as HSPs modulators. In addition, the adaptogens extracted from roots of *Eleutherococcus senticosus*, *Rhodiola rosea*, berry extract from *Schisandra chinensis* are also reported to increase HSP70 when tested in isolated human neuroglia cells [59]. Similarly, ethanolic leave extracts from *Cichorium intybus* and *Jasminum sambac* also induced HSP70 expression in C2C12 myoblasts and rats tissue [60,61]. All these evidences suggested that phytochemical have great impact in prevention of progression of different neurodegenerative diseases.

Curcumin Efficacy in Neurodegenerative Disease Therapy

Polyphenol curcumin, the yellow curry powder of turmeric is derived from the plant *Curcumina longa*. Curcumin has been reported to use since more than five thousand years in traditional Ayurvedic medicine of India, China, Vietnam and other South East Asian countries. Whereas, western world have rediscovered its pleotropic beneficial effects since last two decades only. Several experimental data suggested that it can fight against cancer, heart attacks, strokes, arthritis, diabetes, neurodegenerative diseases and many other modern and chronic deceases [32,38,62-64]. It is also a precious polyphenol used for clearing scars, treating stomachache, detoxifying liver, and can be used for skin beautification. Its anti-amyloid properties suggest that it might be potent drug for the treatment of several neurodegenerative diseases [62-67]. Recent research reports evidenced that curcumin can be used for spectrum of neurological diseases including AD, PD, MS, schizophrenia, depression, epilepsy, cerebral ischemia, brain tumor, and in different neuropathic pain [32,33,38,64] (Figure 2). It can binds and dis-aggregates Aβ oligomers and fibrils and prevents aggregation of this protein in cell free system [63]. Curcumin is anti-inflammatory, antioxidant; stimulate neurogenesis [31,33,38,68]. It has been reported to reduce plaque burden and improve cognitive functions in mouse model of AD, and protected against Aβ-toxicity in vitro and in vivo [69-74]. Recently, we have shown that curcumin reduces HTT aggregates in CAG140KI mouse model of HD [73]. Similarly, Yang et al. revealed that curcumin were able to decreased Aβ and tau aggregates from 3xTg rat and over expressed human tau-Tg mouse model [63]. *In vitro* data showed that out of 214-antioxidant compounds curcumin had the strongest inhibitory effect on the formation of Aβ fibrils [74,75].

Further, Ono et al., have reported that curcumin have a dose dependent effects on the inhibition of Aβ aggregation and destabilize preformed fibrils with an EC50 of 0.09–0.63 μM [76]. Recent experimental data revealed that oral administration of curcumin could inhibit Aβ oligomerization and tau phosphorylation and improve behavioral impairment of AD animal models [31,38,63]. Several other groups also showed that tail vein injection of curcumin for one week had a marked amyloid clearance effect with 30% plaque size reduction in addition to suppression of dystrophic and aberrant neurites [64].

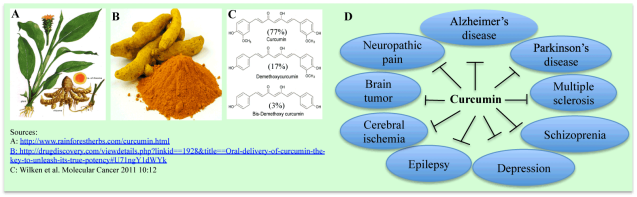

Figure 2: Curcumin source, their derivatives and efficacy in different neurological diseases. A & B: Curcumin derived from root of plant *Curcumin Longa*; C: Major compounds from *Curcumina Longa*; D: Neuroprotective role of curcumin and their derivative in different brain disorders.

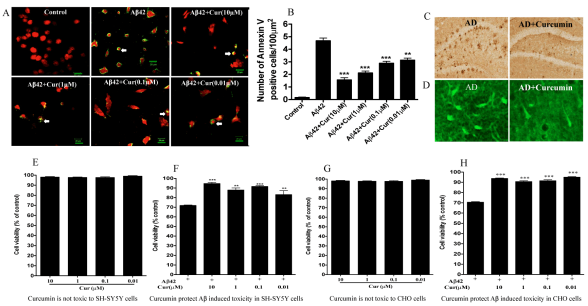

Figure 3: Curcumin inhibits Aβ42 induced neuronal apoptosis in vitro and reduce Aβ-plaque load, neurodegeneration in mouse model of AD. A: SH-SY5Y cells were treated with Aβ42 (10μM) and incubated with different doses of curcumin. After 24h of incubation, cells were stained with Annexin-V tagged with FITC and numbers of apoptotic (white arrow) death were counted. Curcumin protected Aβ42 induced neuronal apoptosis (B). Data are mean ± standard error of mean, $^{**}p<0.01$, $^{***}p<0.001$ compared to Aβ42 treated groups; C & D: Curcumin decreased Aβ-plaque load (hippocampus: 4G8 immunostain) and neurodegeneration (Cortex: Fluoro Jade B staining) in animal model of AD. Figure E-H: Curcumin is safe and having anti-amyloid properties. The Chinese hamster ovary (CHO) (E & F) and SY-SY5Y (G & H) cells were treated with different doses of curcumin for 24h and cell viability were measured by MTT assay. Curcumin showed no toxicity in all these concentrations in both cell lines. Similarly, these cell lines were insulted with Aβ42 (10μM) and treated with same doses of curcumin. After 24h of incubation, curcumin rescued Aβ42 induced cell death in both the cell lines. Results are mean ± standard error of mean, $^{**}p<0.01$, $^{***}p<0.001$ compared to Aβ42 treated group.

Most importantly, binding affinity of curcumin for Aβ aggregates is as high or higher than successful molecular imaging probes such as Pittsburg compound (PIB), Fluorodeoxyglucose (FDG) [77,78]. Curcumin can also reduce Aβ production by inhibiting beta secretase (BACE), the enzyme responsible for synthesis of Aβ from amyloid precursor protein [79-82]. It also decreases Aβ production by inhibiting GSK-3β (the enzyme responsible for phosphorylation of tau) mediated presinilin-1 activation [83]. It can also stimulate phagocytosis of Aβ in a rat AD model [33]. Further, curcumin has been shown to bind with neurofibrillary tangles (NFTs) in human AD

brain and animals [84,85]. It can inhibit tau phosphorylation and aggregation as observed in animal model of AD [74,86]. Similarly, several research groups have shown that curcumin could also bind with other amyloid proteins containing β-pleated sheet structures including α- synuclein [87,88], HTT [35,89] and prion [90] aggregates and inhibits their oligomerization.

Nanotechnology Approaches to Enhance the Bioavailability of Curcumin

Because of its potential impact to prevent and treat a wide spectrum of incurable and chronic diseases, nowadays curcumin is globally accepted as one of the wonder drug for future. Most importantly, it is safe up to 12g/day as seen in animal studies and in phase-I clinical trial [91]. However, despite its pleotropic action against several diseases, the main drawback of successful use of curcumin therapy in clinical trial is its poor solubility and bioavailability [32,92,93]. Basically, most of the free curcumin are instable at cellular pH. After oral administration it becomes glucuronidated in the liver and eliminated from body through urine [94,95]. That is why no free curcumin was detected in plasma from patients in a clinical trial with 2 or 4 g of curcumin administration per day in AD patients [92]. As tissue can absorb very little amount of free curcumin, therefore either high amount of curcumin administration is essential in order to get its beneficial effects or anyhow we need to increase its absorption through elementary system (Figure 4). The main pitfall of use of higher doses of free curcumin, it can destroy normal intestinal flora due to its antibacterial properties, which can increase the risk of indigestion.

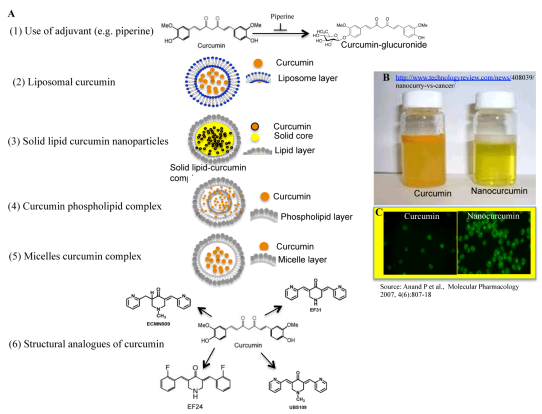

Figure 4: Different nanotechnology strategies to improve curcumin solubility, stability, and bioavailability for its therapeutic use. A: different formulas for making lapidated or nanocurcumin; B: Nanocurcumin increases water solubility compared to regular curcumin; C: Nanocurcumin have greater cell permeability compared to regular one.

Several other commonly reported side effects of use of high doses of curcumin including stomach upset, diarrhea, nausea, allergic reaction and anti-thrombotic activity [96]. Hence, enhancement of curcumin solubility and stability following its oral bioavailability represents a pharmacological challenge for its therapeutic applications. However, numerous approaches have been undertaken to improve the bioavailability of curcumin including nanotechnology-based novel strategies (Table 3). Recently, introduction of most important technologies including use of adjuvant (e.g. piperine), the use of liposomal curcumin (liposome/micelles); solid lipid curcumin nanoparticles; the use of curcumin phospholipid complex; the use of synthetic structural analogues of curcumin (e.g., EF-24), chelating of curcumin with metals, combination with other dietary agents etc [91] have been successfully used to enhance bioavailability of curcumin. These approaches not only increased curcumin bioavailability, but also reduced its cytotoxicity along with increased cellular uptake, enhanced dissolution rates, excellent blood stability etc [36,37,87,97] (Table 3). The adjuvants like piperine is mainly used to interfere with glucuronidation, thus increase prolongation to stay in blood and inhibit its rapid excretion. Whereas liposomes are both hydrophilic and hydrophobic in nature, therefore it is an excellent system for drug delivery, especially curcumin like molecule. The liposome polymers based nanocurcumin have size less than 100 nanometer (nm). It is highly water-soluble, easily absorbed by cell membrane and its bioavailability is increased up to 80-95%, which is 40 times higher than free curcumin [37,91,98].

This formulation appears to be able to achieve this level and is already in clinical trials for cancer, aging as well as AD and other neurodegenerative diseases [37,91,98]. Recently, Bisht et al. have developed a polymer-based nanoparticle called “nanocurcumin”. They have reported that the diameter of this particle is less than 100 nm in size and have similar cellular activity like regular one [36]. Similarly, Tiyaboonchai et al. have developed solid lipid nanoparticles (SLNs) loaded with curcuminoids, which is 450 nm in size and stable for at least 6 months at room temperature. It can release curcumin product slowly up to12 h in vitro [108]. Furthermore, micelles-curcumin formulation has been reported to greatly improved intestinal absorption of curcumin. Ma et al. reported that polymeric micelle-curcumin increase up to 60-fold biological half-life for curcumin in rats compared to use of polyethylene glycol or dextrose solution to solubilize curcumin [109]. Similarly, curcumin-phospholipid formula has been showed a significant improvement in curcumin bioavailability in vivo. Liu et al., reported that when the rat was administered 100 mg/kg of curcumin–phospholipid complex orally, after 2.33 h plasma curcumin level reached to 600 ng/mL, which was two folds more compare to free curcumin. This formula has also increased half-life of curcumin about a 1.5-fold in rat blood [110], whereas Maiti et al., found 3 folds increase with the same formula [37].

Sno		
1	Curcumin nanoparticles	Potential increase antioxidant and anti-hepatoma capacity, improve physicochemical properties of curcumin [99]
2	Antimicrobial curcumin nanoparticles [100]	Enhance antimicrobial activity compared to the regular curcumin

3	Solid lipid nanoparticles curcumin complex	Improve bioavailability and reduce dose (32-155 times) and increase curcumin efficacy [101]
4	Nano crystal solid dispersion of curcumin	Enhanced bioavailability along with high photochemical stability [102]
5	Curcumin phospholipid complex	Increase antioxidant capacity [37]
6	Nano liposome decoated curcumin	Enhance anti amyloid or anti fibrilogenic activity [103]
7	Intravenous injection of curcumin nano-emulsion	Enhance solubility (600 fold), dissolution rate (10 fold) [104]
8	Oil-in water Nano emulsion to encapsulate curcumin	Enhancement of anti-inflammatory activity [37]
9	Cyclodextrin/curcumin self assembly	Improve bioavailability and delivery [105]
10	Curcumin loaded biodegradable polymeric micelles	It is an excellent intravenous injectable aqueous formulation
11	Curcumin loaded hydrogel nanoparticles potential for colon carcinoma [106]	Increase antimalarial action [107]
12	Polymeric nanoparticles encapsulated curcumin (Nanocurcumin)	Effective as the larger amounts of the free compound against pancreatic cancer [36]

Table 3: Advantages of use of different nanotechnology strategies for delivery of curcumin. Application of these technologies improved bioavailability and increases the therapeutic efficacy of curcumin.

In addition, scientist developed isomers or analogues of curcumin, which have similar bioactive capacity like curcumin. For example, EF-24 is a lead analogue of curcumin having antitumor activity *in vitro* and *in vivo*. After oral and intraperitoneal administration, this compound rapidly absorbed and peak plasma concentration reach up to 1μM within 3 min. The bioavailability of this analogue was 60% and 35%, after oral and intraperitoneal administration respectively [111]. Bio-conjugation is another promising technique to enhance curcumin uptake and its bioavailability. Mishra et al. have developed a compound called Biocurcumax (BCM-95), which showed increased bioavailability of 7-8 folds and also bioactivity up to 700% [112]. They have also used curcumin bio-conjugates containing glycine, alanine, and/or piperic acid and found increased cellular permeability and thus enhanced bioavailability [112].

Similarly, since for decade, Cole and Frautschy groups have used different combination of curcumin such as mixed with phosphatidyl choline, olive oil, or stearic acid to increase curcumin solubility and they have found higher levels of curcumin with these formulas in rat's plasma and brain in comparison to regular curcumin [38]. They have analyzed plasma and brain level of curcumin in an animal model and found with 0.1-0.2 μM in plasma and 1-2 μM in brain tissue [38]. Among all these formula, lipidated curcumin used by this groups (Called Longvida developed by Verdure Sciences, Noblesville, IN; www.verduresciences. com) has 11 folds and 4 folds higher plasma and brain level of curcumin respectively compared with equal doses of regular curcumin or curcumin- piperine extracts. Using this lipidated nanoparticle (Longvida), they have shown that this particular formulation decreased Aβ plaque load and improved memory in a mice model of AD [13,38,113]. In another study Cole and Frautschy groups delivered 5 mg lipidated curcumin and achieved more than 2μM brain curcumin levels with in 3h of administration [40]. Similarly, they have found up to 5μM of curcumin in mouse brain tissue after two weeks of feeding of 500-parts per million (ppm) curcumin in chow, suggesting oral delivery of lipidated curcumin can achieve the targeted therapeutic tissue levels of curcumin [38]. This is also reminding that the traditional method of dissolving turmeric in fat during cooking in South East Asian people is likely an effective method to improve its solubility and bioavailability. However, the uses of different lipidated formulas including nanoparticle coated curcumin deliveries are in their beginning stage; need much more development for its successful therapeutic use.

Potential Impact of Nanocurcumin in Activation of Heat Shock System

Despite strong evidence supporting multiple roles of HSPs in amyloid protein degradation [21,24,34,114], knowledge of their dysregulation in different neurodegenerative diseases and their potential as a drug target is unclear. Further, the relationships between the beta-pleated sheet binding of curcumin and removal of these protein aggregates are poorly understood. One possibility is that it is targeting a common endogenous protein clearance pathway, such as the HSP system. Research from Frautschy group support the idea that curcumin administration decreases tau protein aggregation in human tau transgenic (HtauTg) mouse model [13]. They have demonstrated that curcumin has also been shown to reduce soluble tau and increase HSPs in a human tau mouse model [25]. These results indicate that even after tangles are established, tau-dependent dysfunction of the synapses and behavior deficits can be corrected by curcumin treatment [13,25]. Therefore, curcumin might be the promising compounds, which can modulate as well as rectify dysregulation of the heat shock response; their co-chaperones and client proteins in different brain disorders need further investigation.

To test the above hypotheses, we have examined the levels of HSPs and their client proteins (HSP90, HSP70, HSC70, HSP60, HSP40, CDC37, P23, FKBP51 etc.) in AD, tauopathies and HD animal models. For AD animal model, triple transgenic rat (Swedish mutation in APP and mutation in presenilin-1 and presenilin-2) was used. For tauopathies, human tau was over expressed (htau-Tg) in mice, whereas for HD animal model, 140-glutamine codon (CAG) was knocked in (CAG140KI), which produce huge HTT neuropil aggregates and showed significant neurobehavioral impairments [35]. Both

tauopathies and AD animal models showed significant cognitive and neurobehavioral impairments along with significant neuropathological symptoms [13,25]. However, the HD mice were pretreated with 555-ppm regular curcumin and rests two are treated with same doses of solid lipid nanoparticle coated curcumin. The reason for choosing low dose of curcumin is because higher doses e.g. more than 1μM levels of curcumin may actually be less beneficial due to the demonstrated toxic effects in an *in vitro* model of HD [41] and lesser efficacy in reducing amyloid burden in the Tg2576 mouse model of AD [20,25,26]. Further, more than 1μM concentration of curcumin inhibits the proteasome, which may exacerbate the disease, and increased aggregate sizes in vitro in these studies [20,25,26]. However, in our study we been found that there were transgene-dependent reductions of HSPs response along with decline some of their client proteins in all these three neurodegenerative animal models, indicating molecular chaperones are highly affected, whereas, curcumin restored transgenic defects of these proteins (unpublished observation).

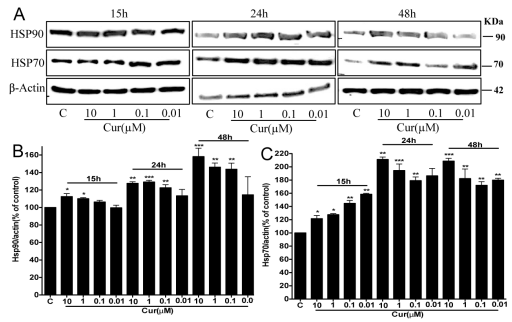

Figure 5: Curcumin induces heat shock response in SH-SY5Y cells. The SH-SY5Y cells were treated with different concentration of curcumin (μM) for 15, 24 and 48 h. After 24 and 48 h of curcumin treatment, HSP90 and HSP70 were significantly increased in compared to control. A: Representative western blot; B & C: Analysis of optical density of HSP90 and HSP70. Data are mean ± standard error of mean, $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ when compared to control.

In this study the transgene dependent loss of HSP70, HSC70 in CAG140KI, htau-Tg mice and 3xTg rat may represent a failure to compensate for aggregate HTT, tau or Aβ oligomers with aging and be relevant to the removal of those toxic aggregates as well as neuroprotection [24,34]. Another line of interest in this context was whether curcumin could modulate HSP90 co-chaperones and their client proteins. Therefore, we have investigated the levels of CDC37, P23 and FKBP51. In general, CDC37 inhibits the HSP90 activity, whereas P23 and immunophillin FKBP51 activates it. The immunophillin FKBP51 is a mitochondrial protein that translocate to the nucleus to protect cells against oxidative stress [115], and its level has been reported to reduce in AD brain [116]. It has been observed that CDC37, P23 and FKBP51 were significantly reduced in all the three animal models, suggesting that there was dysregulation of HSP90 co-chaperones, whereas curcumin treatment ameliorated their levels. In addition, to determine whether curcumin can induce the heat shock response in control cells, we have treated CHO and SH-SY5Y cell lines with four different doses (μM: 10, 1, 0.1 and 0.01) of curcumin. It is observed that even 0.01 μM of curcumin was able to increase HSP90 and HSP70 after 24 h of incubation in SH-SY5Y cells (Figure 5). The same doses of curcumin were tested for their toxicity and Aβ42 induced cell death in CHO and SH- SY5Y cell lines, and it has been observed that all these concentrations of curcumin protected Aβ42 induced apoptosis (Figure 3). These observations indicate that very low amount of curcumin may be required for neuroprotection as well as to activate cellular HSS, but definitely need further investigations to confirm these findings.

Conclusion

Accumulation of misfolded proteins in the intra and extracellular spaces of the central nervous system are the leading cause of synaptic loss, neurodegeneration, and cognitive and behavioral impairment in several brain diseases. Endogenous protein clearance pathway such as HSPs have significant role in protein folding and maturation, and renaturation of misfolded proteins, thus play pivotal role to remove these aggregated proteins. This essential system is significantly down regulated in different brain diseases. Maintenance or activation of this system using drug/molecules/compound would be a great strategy to remove these toxic aggregates. Recently, anti-amyloid polyphenol curcumin have been found to be an ameliorative role against amyloid induced dysregulation of HSS. Increase solubility, stability and bioavailability of curcumin by nanocurcumin formulation are the promising strategies for restoration and up-regulation of HSS to rectify the deleterious effect in several neurodegenerative diseases caused by misfolded proteins accumulation.

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Conflict of interest

There is no conflict of interests.

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