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Heat shock treatment reduces beta amyloid toxicity *in vivo* by diminishing oligomers

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Abstract

Heat shock response, mediated by heat shock proteins, is a highly conserved physiological process in multicellular organisms for reestablishment of cellular homeostasis. Expression of heat shock factors and subsequent heat shock protein plays a role in protection against proteotoxicity in invertebrate and vertebrate models. Proteotoxicity due to β -amyloid peptide ($A\beta$) oligomerization has been linked to the pathogenesis of Alzheimer's disease. Previously, we demonstrated that progressive paralysis induced by expression of human $A\beta_{1-42}$ in transgenic *C. elegans* was alleviated by $A\beta$ oligomer inhibitors *ginkgo biloba* extract and its constituents (Wu, et al., J. Neurosci 2006, 26:13102-13). In this study, we apply a protective heat shock to the transgenic *C. elegans* and demonstrate: 1) a remarkable delay in paralysis, 2) increased expression of small heat shock protein HSP16.2, and 3) significant reduction of $A\beta$ oligomers in a heat shock time-dependent manner. These results suggest that transient heat shock lessens $A\beta$ toxicity by diminishing $A\beta$ oligomerization, which provide a link between up regulation of endogenous chaperone proteins and protection against $A\beta$ proteotoxicity *in vivo*.

Keywords

heat shock; $A\beta$ oligomers; Alzheimer's disease; *C. elegans*

Introduction

The “amyloid cascade hypothesis” predicts that the progression of pathogenesis of Alzheimer's disease (AD) involves multiple factors such as amyloid aggregation, oxidative

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stress, loss of ion homeostasis, inflammation and subsequent neuronal death (Hardy et al., 2002). The intermediate A β oligomers have been shown to be the toxic species in the early stage of AD (Klein et al., 2001; Walsh et al., 2002). In a transgenic *C. elegans* model of AD, intracellular expression of human A β is associated with elevated oxidative stress (Boyd-Kimball et al., 2006), accumulation of A β aggregation and progressive paralysis behavior (Link 2005). Small heat shock protein HSP16.2 has been reported to co-localize with A β in the transgenic *C. elegans* (Fonte et al., 2002), and its over expression suppressed the paralysis (Fonte et al., 2007). However, the functional link between HSP and A β on protection against A β toxicity remains unknown. Heat shock response in *C. elegans* is a neuronal controlled behavior (Prahlad et al., 2008). Transcription factor HSF-1, which regulates heat shock response by up regulation of heat shock proteins in *C. elegans*, has been coupled to normal aging and age-related diseases (Hsu et al., 2003). Recently, it also has been associated with dietary restriction (Steinkraus et al., 2008) and insulin-like signaling (Cohen et al., 2006) in protection against proteotoxicity. Previously we reported that A β -induced paralysis was reduced by feeding the A β worms with agents that inhibit A β oligomers, but not with known antioxidants (Wu et al., 2006). In this study, we apply a protective heat shock treatment (2h, 35°C) to A β *C. elegans* and reveal a significant decrease of A β toxicity and A β oligomers in the transgenic worms. This data is consistent with the view that co-expression of HSP16.2 or other factors induced by heat shock in A β *C. elegans* lessened A β toxicity in these worms (Fonte et al. 2007). In addition, it provides new insight into the role of non-invasive physical treatment and endogenous chaperone proteins in regulation of A β aggregation and toxicity.

Materials and Methods

C. elegans strains

The transgenic strain CL4176 (Link et al., 2005) expresses intracellular muscle- specific human A β ₁₋₄₂. Synchronized eggs were cultured on fresh NGM plates at 16°C in a temperature-controlled incubator (Sheldon, Cornelius, OR).

Paralysis assays

A β transgene expression was induced at the 36th h after hatching by up-shifting the temperature from 16°C to 23 °C. The number of paralyzed worms (100 in each treatment group) was scored at one-hour interval until the last one paralyzed (Wu et al. 2006).

Heat shock was given to CL4176 strain at 24th hour after temperature up-shift, by elevating temperature from 23 °C to 35 °C for 2h, followed by returning to 23°C.

H₂O₂ assay in C. elegans was preformed as previously described (Smith et al., 2003). Age-synchronized A β worms (CL4176) were collected at 36 h after temperature up-shift in groups of 40 worms. The worms were subjected to sonication and were collected into 96-well plates.

DCF-DA (50 μ M) was added to each well for quantification of fluorescence in a Microplate Fluorescence Reader (Bio-Tek, Winooski, VT) at the excitation at 485 nm and emission at 530 nm. *Western blotting of HSP16.2 and A β species* Following heat shock treatments, the worms were homogenized in the cell lysis buffer (25mM Tris 7.5, 5 mM NaCl, 1mM DTT, 5mMEDTA) with protease inhibitor cocktail (Sigma, Saint Louis, MO). Equal amounts of the total protein (40 μ g) were loaded in each lane. Antibody to HSP16.2 was a gift from Dr. C. Link (Fonte et al. 2007). Antibody to A β ₁₋₁₇ (6E10, at 1:1000 dilutions) was from Signet (Dedham, MA). Antibody specific to A β oligomers (NU4, 1:1000) was generated in W. Klein's lab (Lambert et al., 2007). Anti-mouse IgG or anti-rabbit IgG, (1:5000, Signet) were the secondary antibodies. Mean densities of the A β oligomers were analyzed by a gel

documentation system (Alpha innotech, Imgen Technologies, CA). *Statistical analyses* Differences between untreated and heat shock treated groups were analyzed for statistical significance by Independent Student's *t* Test of two groups using Microsoft Origin 6.0 software (Northampton, MA). P-value < 0.05 is considered statistically significant.

Results

Protective heat shock remarkably delays paralysis in the transgenic *C. elegans*

We first examined the effect of protective heat shock on A β -induced toxicity in a transgenic *C. elegans* model of AD (strain CL4176). The A β worms were heat shocked for 2h at 35°C followed by the paralysis assay. Time course of paralysis revealed that heat shock treatment (HS, filled squares) significantly deferred paralysis compared with non-heat shocked worms (no HS, open circles, Fig 1A). The delayed paralysis was not additive by co-treatment with HS and EGb 761 (100 μ g/ml), a *ginkgo biloba* leave extract that reduced paralysis by inhibiting A β oligomers (Wu et al., 2006), compared with either treatment alone (data not shown), suggesting a share mechanism of action. To elucidate the biochemical consequence of heat shock, the levels of H₂O₂ were measured by DCF assay. As previously reported, the levels of H₂O₂ were elevated in A β worms (Smith et al. 2003), and the effect of heat shock treatment (+HS) significantly opposed the level of H₂O₂ in both *C. elegans* strains (A β worms) and the non-transgenic control worms (Ctrl worms) compared with no heat shocked controls (-HS, Fig.1B, **p*<0.05; ***p*<0.01; *n*=3). We then determined whether heat shock could induce HSP16.2 over expression using Western blotting in the worms after increasing time of heat shock treatment. A notable enhancement of HSP16.2 expression was observed in the A β worms following 2 h heat shock (Fig.2A, arrow on the right).

Protective heat shock significantly reduces A β oligomers in transgenic *C. elegans*

A β species were detected using either an antibody specific to A β oligomers (NU4) or to total A β (6E10). Heat shock treatment in A β worms considerably reduced several A β oligomeric species such as 20 kD and 100 kD as shown with arrows (Fig.2B, A β species NU4), but not total A β (Fig.2C, A β species 6E10), in a time dependent manner. This suggests that heat shock specifically inhibits toxic A β oligomers rather than A β transgene expression in the worms. Quantitative analysis of A β oligomers (NU4) illustrate (Fig. 2D) that heat shock for 2h (HS) significantly decreased the levels of A β oilgomers (100 kD) in comparison with the untreated controls (-HS 100% vs. HS 57.7 $\pm$ 11.4%, *P*=0.003, *n*=3). Although wild type worms (Ctrl worms) also exhibited background NU4 immunoreactivity, heat shock treatment did not affect this level (Fig.2D), signifying specificity to the A β oligomers by the heat shock.

Discussion

We have revealed that heat shock remarkably reduced A β toxicity in A β worms (Fig. 1A&2A). The result is consistent with a recent report demonstrating that co-expression of HSP16.2 or other factors induced by heat shock in A β worms reduced their paralysis (Fonte et al. 2007). The most important finding in this study is that protective heat shock is associated with reduction of A β oligomeric protein, but not A β transgene expression (Fig. 2B&C). Although the mechanism of the interaction between HSP and A β on A β toxicity remains unclear, the HSP family in *C. elegans* has been shown to have chaperone activity *in vitro* (Hinault et al., 2006) and is homologous to mammalian α B crystalline (Link et al. 2005). The mechanism may also share some common features as EGb 761 which binds to A β *in vitro* and alleviates A β toxicity *in vivo* (Wu et al. 2006). It is possible that activation of multiple members of the HSP family by heat shock treatment is necessary for degradation of A β oligomers because heat-shock treatment is known to induce HSF-1 and several heat

shock proteins including hsp 70 and hsp16.2 (Pahlad et al., 2008). This may explain undetectable changes of A β oligomers by genetic co-expression of HSP16.2 alone in the transgenic *C. elegans* (Fonte et al. 2007). Interestingly, reduction of polyQ-mediated Huntingtin toxicity was reduced in the worms which required HSF-1 (Steinkraus et al. 2008), and in mammalian cells by 17-DMAG that induces HSP (Herbst et al., 2007). Protection by heat shock proteins may hold true for mammals; that is, up regulation of a stress response is therapeutically beneficial to health and disease. It has been shown that extended exposure to extreme stress is damaging while transient exposure to elevated temperature can generate a cross-protective effect against constant lethal and/or pathological stimulation (Morimoto et al., 1998; Mattson 2004). Taken together, these data implicate that up-regulation of an endogenous defense system by thermal or physical activities would be a potentially useful therapy for Alzheimer's disease.

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Figure 1. Aβ-induced paralysis (**A**) and levels of intracellular H₂O₂ (**B**) in transgenic *C. elegans* strain (Aβ worms) or wild type (Ctrl strain) with (+HS) or without (-HS) heat shock for 2h at 35°C. Each group contains 100 worms. Results were from three independent tests. * $P < 0.05$, ** $P < 0.01$.

Figure 2.

Western blots of small heat shock protein HSP16.2 (A), Aβ oligomers (antibody NU4) (B) or total Aβ (antibody 6E10) (C) in Aβ worms treated with increasing time of heat shock (0-5h). D. Mean density of Aβ oligomers (NU4) in wild type (Ctrl worms) or Aβ worms, untreated (-HS) or heat shocked (HS) for 2h. Data were obtained from three independent experiments (** $P < 0.01$).