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Research report

Hsp27 and Hsp70 administered in combination have a potent protective effect against FALS-associated SOD1-mutant-induced cell death in mammalian neuronal cells

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Abstract

Amyotrophic lateral sclerosis (ALS) is an adult-onset degenerative disorder characterised by the death of motor neurons in the cortex, brainstem, and spinal cord; resulting in progressive muscle weakness, atrophy, and death from respiratory paralysis, usually within 3–5 years of symptom onset. Approximately 10% of ALS cases are familial (FALS). Mutations in superoxide dismutase-1 (SOD1) cause approximately 20% of FALS cases and there is overwhelming evidence that a toxic gain of function is the cause of the disease. We have previously shown that FALS-associated SOD1 disease mutants enhanced neuronal death in response to a wide range of stimuli tested whereas wt-SOD1 protected against all insults. We demonstrate for the first time that over-expression of either heat shock protein Hsp27 or Hsp70 has a protective effect against SOD1 disease associated mutant-induced cell death. However, over-expression of Hsp27 and Hsp70 together has a greater potent anti-apoptotic effect, than when expressed singly, against the damaging effects of mutant SOD1. Our results indicate that FALS-associated SOD1 disease mutants possess enhanced death-inducing properties and lead to increased apoptosis which can be prevented by either the use of specific caspase inhibitors or Hsp27 and/or Hsp70 over-expression. This potent protective effect of Hsp27 and Hsp70 against the FALS-associated SOD1 disease mutants may be of potential therapeutic importance.
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Theme: Disorders of the nervous system
Topic: Degenerative disease

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset degenerative disorder characterised by the death of motor neurons in the cortex, brainstem, and spinal cord [21] with the result being progressive muscle weakness, atrophy, and death from respiratory paralysis, usually within 3–5 years of symptom onset [5,6]. The age-adjusted worldwide incidence of ALS is between 1 and 3 per 100,000 person years and increases with age (disregarding any obvious race-related differences) [1,47,48]. The crude prevalence of ALS is estimated at between 4 and 6 per 100,000 population [1,47,70]. Approximately 10% of ALS cases are familial (FALS) [13]. Mutations in superoxide dismutase-1 (SOD1) cause approximately 20% of FALS cases [33,65] and there

Abbreviations: ALS, amyotrophic lateral sclerosis; SOD1, Cu/Zn-superoxide dismutase-1; SOD2, Mn-superoxide dismutase-2; Hsps, heat shock proteins; DRG, dorsal root ganglia; TUNEL, terminal dUTP nick end labelling; wt, wild type

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is overwhelming evidence that a toxic gain of function in the SOD1 mutants is the cause of the disease [72,76]. However, it remains unclear as to how mutant SOD1 causes such selective motor neuron death.

SOD1 has also been intensively investigated due to its biological importance as an antioxidant. SOD1 catalyses the conversion of superoxide to H_2O_2 and glutathione peroxidase and catalase then converts H_2O_2 to water [7]. SOD1 is a 153-amino-acid, 16-kDa protein that functions as a homodimer and comprises approximately 1% of total cytosolic protein [4] and is highly conserved through evolution and constitutively expressed in all cells in eukaryotes [7]. In addition to dismutase activity, SOD1 also has a peroxidase function, catalysing oxidative reactions of substrates by hydrogen peroxide.

We recently obtained evidence demonstrating that wt-SOD1 can protect neuronal cells against a wide range of death inducing stimuli [59] which has been demonstrated previously in several studies [30,63]. In contrast, we also found that the disease-associated SOD1 mutants G93A and G93R enhanced cell death in response to all stimuli [59].

Having established these effects, we undertook in the present project to study the potential neuroprotective/therapeutic effects of various heat shock proteins (hsps) in this well characterised neuronal system, by means of a highly efficient HSV-based gene delivery system [71]. Accumulation of abnormally folded proteins in the nucleus or the cytosol that occurs as a result of stress such as elevated temperature, free oxygen radicals, heavy metals and even antibiotics [68], results in the formation of aggregates that disturb normal cellular function and trigger cell death [68]. Such a mechanism has been implicated in the pathogenesis of lesions that characterise many neurodegenerative diseases. Neuronal cells seem to particularly vulnerable to the toxic effects of mutant or misfolded protein aggregates. Most Hsps are involved in the proper folding and/or elimination of misfolded protein, thus acting as the first line of defence against it and contributing to cell survival.

SOD1 is as mentioned previously a ubiquitously expressed protein and there is no change in its abundance in motor neurons. In SOD1 mutant cells, however, there is a reduction in the activity of molecular chaperones which results in the accumulation and inefficient removal of mutant SOD1 [8]. The mutated protein may be unstable so that it precipitates to generate toxic aggregates [5,6]. This is important when taking into account that SOD1 represents approximately 1% of cytosolic protein. Changes in folding, solubility, or degradation of such an abundant protein may possibly result in aggregates, consistent with the detection of SOD1 immunoreactive inclusion bodies in motor neurons expressing mutant SOD1 but not with wild-type SOD1 [4,10,23]. The formation of mutant SOD1 aggregates is prevented by induction of stress-inducible heatshock protein Hsp70 in cultured neuronal cells, suggesting that the decrease in the pool of available chaperones leaves mutant SOD1 cells particularly susceptible to physiological and environmental stress [8].

It remains controversial as to whether cytoplasmic mutant SOD1 aggregates are toxic [10] or not [19,37,39]. Previous studies in neuronal cells and cultured primary motor neurons have demonstrated that the inhibition of cytoplasmic aggregate formation by induction of heat shock protein Hsp70 assured cell survival at an early stage but was not able to prevent eventual cell death at the late stage in the in vitro models of FALS [8,69].

Some of the important house-keeping functions accredited to the molecular chaperones include the import of proteins into cellular compartments; folding of proteins in the cytosol, endoplasmic reticulum, and mitochondria; degradation of unstable proteins; dissolution of protein complexes; prevention of protein aggregation; control of regulatory proteins; and refolding of misfolded proteins [11]. The main inducible Hsps of the nervous system are Hsp27 and Hsp70 and both have been shown to be neuroprotective. In particular, over-expression of Hsp27 in cultured neuronal cells provides protection from induction of apoptosis by various stimuli such as neurotrophic factor or serum removal [71]. Hsp70 enhances the ability of cells to cope with increased concentrations of unfolded or denatured proteins, [53] and is protective against a wide range of lethal stimuli.

Hsp27 expression is seen to correlate with increased survival in response to cytotoxic stimuli and has been shown to prevent cell death by a wide variety of agents that cause apoptosis [3,71]. Over the years, evidence has accumulated to show that Hsp27 can inhibit apoptosis through a direct inhibition of caspase activation [27,67]. It is thought that as a molecular chaperone, it is not implausible that Hsp27 may regulate the activation of caspases through an ability to interact with one or more components of the apoptosome complex [17]. In addition, expression of Hsp27 is also associated with inhibition of apoptosis initiated by the binding of death ligands to cell surface receptors such as Fas [50].

In the past, we have constructed HSV-based vectors expressing individual hsps and used them to investigate their protective effects both in vitro and in vivo [38,71]. We have also constructed HSV-based vectors expressing wt-SOD1, G93R-SOD1 mutant and control GFP as described previously [59]. These viruses offer a highly effective means of specific gene delivery to primary neuronal cells as well as ND7 cells which are immortalised cells derived from sensory neurons, with a transduction efficiency of over 90%. Although studies have examined the protective effects of over-expression of Hsps, none have investigated conclusively the protective effects of Hsp27 or Hsp70 over-expression, or combined effects of both together, with respect to FALS-associated SOD1 mutants. In the present project, these Hsp-expressing viruses were used in order to ascertain whether Hsp27 or Hsp70 either singly or in combination, have any protective effect against a range of stresses, applied in a well characterised in vitro system, in

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