Molecular chaperones as therapeutic targets in amyotrophic lateral sclerosis

B. Kalmar, D. Kieran1 and L. Greensmith2
Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College London, Queen Square, London WC1N 3BG, U.K.

Abstract
Neurodegenerative diseases are characterized by a number of common hallmarks, such as the presence of intracellular aggregates and activation of the apoptotic cell-death pathway. Intracellular chaperones, responsible for protein integrity and structural repair, may play a crucial role in the progression of a disease. In this paper, we aim to summarize our understanding of the role and potential of a particular family of chaperones, the heat-shock proteins, in neurodegeneration, by focusing our discussion on models of motoneuron death.

Introduction
Neurodegenerative diseases are characterized by changes in protein structure, which result in misfolding and aggregation. Molecular chaperones such as hsps (heat-shock proteins) are thought to play a critical role in preventing the accumulation and aggregation of misfolded proteins [1]. Almost all hsp families have members that are constitutively and ubiquitously expressed in all eukaryotic cells, the so-called heat-shock cognates. These proteins are involved in housekeeping roles, such as protein synthesis, transport and signal transduction. However, the expression of other members of the same hsp family can be induced by stress. These stress-induced hsps play a crucial role in the recovery of proteins following exposure to cellular stress. Thus damaged proteins that lose their structural integrity may be assisted by hsps to maintain or re-establish their functional structure as part of the heat-shock response. This endogenous cellular defence system can be activated by a variety of stressful stimuli, and is characterized by the activation of heat-shock genes by the stress-related transcription factor, HSF-1 [2]. In addition to this chaperoning role, hsps can also interfere with apoptosis, and a number of hsps have been shown to act as negative regulators of the apoptotic cascade [3].

Thus hsps have a dual functionality and can act both as modulators of survival and death-signalling pathways and as protein chaperones. They are therefore an attractive choice for therapeutic targeting in neurodegenerative diseases. In this paper, we present evidence that pharmacological enhancement of the heat-shock response is a successful neuroprotective strategy using models of motoneuron degeneration.

Role of hsps in motoneuron survival following peripheral nerve injury
Injury to the sciatic nerve of neonatal rats is a well-characterized model of acute motoneuron degeneration in which up to 90% of the sciatic motoneurons die by apoptosis, usually within 24–48 h of injury [4]. However, the same injury inflicted at 1 week of age results in almost no motoneuron loss. Thus dramatic changes occur within motoneurons during the first week of post-natal development which render motoneurons resistant to the effects of nerve injury. During this period, there is an increase in the number of motoneurons that express hsp27 [5]. Following nerve injury, a greater proportion of motoneurons that survive are hsp27-immunoreactive than in age-matched uninjured spinal cords. Moreover, motoneurons that express hsp27 appear to be more resistant to apoptosis, since double-labelling immunofluorescence studies show that injured motoneurons do not co-express markers of apoptosis and hsp27. These results suggest that either hsp27 enables motoneurons to resist injury or that the injury itself induces premature expression of this hsp [5]. However, analysis of the time course of expression of hsp27 and apoptotic markers such as activated caspase 3 reveals that although apoptosis occurs rapidly within 24 h of injury, hsp27 expression only increases several days after injury. Thus many motoneurons appear to die before they are able to up-regulate hsp27.

These observations suggest that hsp27 may prevent apoptosis in injured motoneurons [6]. We tested this possibility directly in an in vitro model of motoneuron degeneration. Primary motoneurons in culture were exposed to fluorescently labelled hsp27 protein, which was taken up by motoneurons within 1 h of incubation. The effect of treatment with hsp27 on the vulnerability of motoneurons to apoptotic stimuli, including staurosporine, H2O2 and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) was tested. Our results show that hsp27-treated motoneurons were significantly more resistant to oxidative and...
proapoptotic insults, suggesting that increased hsp27 expression in motoneurons can protect them from a wide variety of insults.

We have also examined the expression of other hsps and found that, in contrast with hsp27, hsps such as hsp70 and hsp90 are not developmentally regulated in and around the motoneurons [5]. Hsp70 is expressed in motoneurons at a very low level throughout development. Hsp90 is strongly expressed during embryonic and post-natal development, possibly because it plays a role in the formation of intracellular receptor complexes. Injury to the sciatic nerve has little if any effect on the expression of these hsps in motoneurons, which is surprising in view of their well-characterized cytoprotective effects [3,7]. However, recent work has shown that motoneurons have an unusually high threshold for the activation of HSF-1, the major stress-sensing transcription factor that regulates hsp expression [8].

Arimoclomol is a member of a group of synthetic hydroxylamine derivatives that act as pharmacological co-inducers of hsps in many cell types [9]. We have shown that treatment with arimoclomol rescues motoneurons in vivo from injury-induced cell death [10]. Moreover, levels of hsp70 and hsp90 are significantly increased in the spinal cords of treated animals, but not within motoneurons. Double-immunofluorescence shows that arimoclomol induces a significant increase in hsp70 in glial cells surrounding the injured motoneurons, which are co-immunoreactive for hsp70 and GFAP (glial fibrillary acidic protein), an astroglial marker. Thus treatment with arimoclomol enhances the heat-shock response in astroglia, which in turn increases the resistance of motoneurons to the cytotoxic effects of nerve injury [10].

The therapeutic potential of small synthetic hsp co-inducers in ALS (amyotrophic lateral sclerosis)

Approx. 20% of familial ALS cases are due to mutations in the gene encoding SOD1 (superoxide dismutase) [11]. Transgenic mice expressing the mutant human SOD1 gene develop pathology and disease signs that reflect those observed in ALS patients. In these mice, motoneurons die progressively from approx. 75 days until death at approx. 130 days and therefore model chronic motoneuron degeneration.

The heat-shock response has been studied both in vivo in SOD1 mice and in vitro in motoneurons transfected with mutant SOD1. In spinal cords of SOD1 mice, hsp25 (the mouse equivalent of human hsp27) increases during the later stages of disease, predominantly in reactive glial cells [12]. This late-stage induction of hsp25 is preceded by a marked decrease of levels in late presymptomatic mice, suggesting a role for the small hsps family in disease pathology [13]. Hsp70 expression also increases during disease progression [14]. In vitro, in SOD1-transfected cells, hsp70 overexpression reduces aggregate formation and increases resistance to stressful stimuli [15]. However, under physiological conditions in vivo, the high threshold for activation of the heat-shock response in motoneurons renders these cells intrinsically less capable of reacting to cellular stress in a timely and co-ordinated manner [8].

We have examined the possibility that treatment of SOD1 mice with co-inducers of the heat-shock response will overcome this intrinsic inability of motoneurons to induce the heat-shock response and rescue motoneurons. These compounds bind to HSF-1 and prolong its binding to the heat-shock element of hsp gene promoters, resulting in increased hsp transcription. Our results have shown that treatment of SOD1 mice with arimoclomol significantly increases lifespan, delays disease progression and rescues motoneurons [14]. Moreover, these improvements are observed when treatment is initiated either presymptomatically or post-symptomatically. Treatment with arimoclomol induces an increase in HSF-1 phosphorylation in the spinal cord, followed by an increased expression of hsp70 and hsp90 in both astroglial cells and motoneurons. These results are in contrast with the effects of arimoclomol on acute-injury-induced motoneuron death, in which hsp expression was enhanced only in glial cells.

In conclusion, our results show that arimoclomol has neuroprotective effects on both acute and chronic models of motoneuron degeneration and suggest that treatment with hsp co-inducers may also prove beneficial in ALS and other neurodegenerative diseases.

References

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