Heat-Shock Proteins in Clinical Neurology

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Heat-Shock Proteins (HSPs) are antigen-presenting protein-aggregation-preventing chaperones, induced by cellular stress in eukaryotic cells. In this review, we focus on recent HSP advances in neurological disorders. In myasthenia gravis, patients responding to immunosuppressive therapy have reduced serum HSP-71 antibodies. Generalized and ocular myasthenia gravis patients have elevated serum HSP-70 antibodies, indicating common pathogenic mechanisms. In Guillain-Barré syndrome, HSP-70 antibodies are elevated in serum and cerebrospinal fluid, and serum levels are higher than in myasthenia gravis and multiple sclerosis. In multiple sclerosis, serum HSP-27 antibodies are elevated during relapses providing disease activation marker, while \(\alpha,\beta\)-crystallin expression in brain lesions indicates remission phase initiation. In acute stroke, serum HSP-27 antibodies are elevated irrespective of stroke type and duration. In epilepsy, HSP-27 is induced in patients’ astrocytes and cerebral blood vessel walls, and \(\alpha,\beta\)-crystallin is expressed in epileptic foci. In neurodegenerative disorders such as Alzheimer dementia and Parkinson’s disease, HSPs are upregulated in brain tissue, and \(\alpha,\beta\)-crystallin modulates superoxide dismutase-1 (SOD-1) tissue accumulation in familial amyotrophic lateral sclerosis. HSPs play an important role in antigen-presentation and tolerance development. Antibody-mediated interference with their function alters immune responses causing neuropahtology. The role of HSPs in clinical neurology should be the subject of future investigation.

Key Words
Heat-shock proteins • Myasthenia gravis • Guillain-Barré syndrome • Multiple sclerosis • Stroke • Epilepsy

Abstract
Heat-shock proteins (HSPs) are antigen-presenting protein-aggregation-preventing chaperones, induced by cellular stress in eukaryotic cells. In this review, we focus on recent HSP advances in neurological disorders. In myasthenia gravis, patients responding to immunosuppressive therapy have reduced serum HSP-71 antibodies. Generalized and ocular myasthenia gravis patients have elevated serum HSP-70 antibodies, indicating common pathogenic mechanisms. In Guillain-Barré syndrome, HSP-70 antibodies are elevated in serum and cerebrospinal fluid, and serum levels are higher than in myasthenia gravis and multiple sclerosis. In multiple sclerosis, serum HSP-27 antibodies are elevated during relapses providing disease activation marker, while \(\alpha,\beta\)-crystallin expression in brain lesions indicates remission phase initiation. In acute stroke, serum HSP-27 antibodies are elevated irrespective of stroke type and duration. In epilepsy, HSP-27 is induced in patients’ astrocytes and cerebral blood vessel walls, and \(\alpha,\beta\)-crystallin is expressed in epileptic foci. In neurodegenerative disorders such as Alzheimer dementia and Parkinson’s disease, HSPs are upregulated in brain tissue, and \(\alpha,\beta\)-crystallin modulates superoxide dismutase-1 (SOD-1) tissue accumulation in familial amyotrophic lateral sclerosis. HSPs play an important role in antigen-presentation and tolerance development. Antibody-mediated interference with their function alters immune responses causing neuropathology. The role of HSPs in clinical neurology should be the subject of future investigation.

Heat-Shock Proteins
Heat-shock proteins (HSPs) are divided into subfamilies according to weight [1]. They play a crucial role in functioning as chaperones to prevent protein misfolding and aggregation [2]. HSPs are expressed at low levels in most eukaryotic cells, but are induced by cellular stress such as increased temperature, radiation, exposure to chemicals, oxidative stress and various physiological and pathological stimuli [1–3]. In addition to being chaperone proteins, the HSPs play a part in antigen presentation and cross-presentation [4]. They also function as cytokines to induce production of proinflammatory cytokines and promote dendritic cell maturation [5, 6].

Antibodies targeting HSP-70 have been detected in sera from patients with sensory neuronal hearing loss [7], systemic lupus erythematosus [8], and juvenile idiopathic arthritis [9]. When applying HSP-70 antibodies together with an agent inducing allergic dermatitis, mice did not develop the disease but gained systemic antigen-
specific tolerance, which could also be adoptively transferred [10]. HSP-70 is believed to be involved in the pathogenesis of several autoimmune disorders, including Behçet’s disease [11], Grave’s disease [12, 13], and increased levels are found locally in experimental autoimmune neuritis [14]. Its ability to augment antigen presentation has been shown in experimentally induced diabetes mellitus [15], HSP-70 therefore plays a role in antigen presentation and development of tolerance, and antibody-mediated interference with its function can alter immune responses.

A pathogenic role of HSPs has been suggested for some neurological disorders, and advances have been made in understanding this role. HSPs may even have neuroprotective properties [16]. The aim of this paper is to review the field, focusing on possible current and future HSP implications for neurological disorders.

### HSPs in Myasthenia Gravis

Myasthenia gravis (MG) is an autoimmune disease with skeletal muscle weakness. Autoantibodies against acetylcholine receptor (AChR) in the postsynaptic membrane are present in 85% of generalized MG (GMG) patients and in half of ocular MG (OMG) patients [17, 18]. While 15% of OMG patients remain purely ocular, many patients develop GMG during the first 2 years of the disease [19]. Thymoma is present in 15% of MG patients [20]. AChR autoantibodies impair neuromuscular transmission by cross-linking AChR and increasing its degradation, and also by complement-mediated postsynaptic membrane damage, and direct blockade of ligand-receptor interaction [17, 21]. Antibodies to muscle-specific kinase (MuSK) are present in a proportion of AChR antibody-negative MG patients [22, 23]. MuSK is a signaling protein controlling AChR clustering and formation of the neuromuscular junction, triggered by agrin interaction with MuSK [24, 25]. MG without detectable antibodies to AChR and MuSK is called seronegative MG. Other antibodies are present in MG, such as antibodies to titin, ryanodine receptor, myosin, and α-actin. These antibodies are associated with MG subtype and disease severity [26].

HSPs represent a novel autoimmune target in MG. MG patients who responded well to immunosuppressive therapy had significantly reduced HSP-71 antibody levels compared to those who did not respond to the same therapy [27]. Measuring HSP-71 antibodies in MG patients’ sera could therefore serve as a marker of therapy efficacy. Both GMG and OMG patients have higher HSP-70 antibody concentrations compared to healthy controls [28], and irrespective of age, gender, or AChR antibody status. Higher levels of HSP-70 antibodies are likely to reflect increased exposure to HSP-70 antigens, current or previous, in the circulation or bound to cell membranes [28]. OMG affects a few small muscles only, and patients experience mild and focal symptoms. It is interesting that OMG patients still have significantly increased HSP-70 antibody levels in their sera, even though AChR antibodies are not detectable. This supports the notion that OMG, similarly to GMG, is a generalized autoimmune disease, and that common pathogenic mechanisms are implicated for these two disease entities.

### HSPs in Guillain-Barré Syndrome

Increased HSP-70 antibody levels have been reported in the cerebrospinal fluid from patients with acute and untreated Guillain-Barré syndrome when compared to patients with motor neuron disease [29]. In a recent study [29], it was demonstrated that Guillain-Barré syndrome patients had elevated serum HSP-70 antibody concentrations compared to healthy controls, MG patients, and multiple sclerosis (MS) patients. The previous study [29] did not find this difference in serum HSP-70 concentration – perhaps because only serum IgG levels were measured, whereas in the recent study, antibodies of all classes (IgG, IgA and IgM) were included.

### HSPs in Multiple Sclerosis

An increased occurrence of T-cell lines recognizing HSP-70 antigen in MS patients was reported many years ago, indicating a role for HSP-70 in the pathogenesis of MS [30]. In the mean time, other HSP antigens and antibodies emerged in the pathogenesis of MS, such as the demonstration of a marked elevation of serum HSP-27 antibodies during the relapsing phase of MS [31]. HSP-27 antibodies may serve as a marker of disease activation if these results can be confirmed by others. Another HSP, α,β-crystallin, is present in the cerebrospinal fluid of MS patients [32]. However, antibodies to α,β-crystallin are found in the serum of both MS patients and healthy controls [33]. The role of such antibodies is not clear. In tissue microarrays, α,β-crystallin showed increased expression in MS brain tissue compared with controls independent of demyelination, indicating progressive α,β-crystallin
upregulation of expression [34]. It has turned out that α,β-crystallin is a key regulatory molecule with inflammation-inhibiting properties in relapsing-remitting MS. The expression of this molecule in MS lesions indicates the initiation of the remission phase [35].

**HSPs in Stroke**

In a recent study, Azarpazhooh et al. [36] reported that serum HSP-27 antibody levels measured 24 h after the onset of stroke were significantly higher than in controls, but did not differ among patients with different stroke types and did not predict the 6-month prognosis.

In rats subjected to ischemia, a significant upregulation of HSP-70 after 3 weeks of exercise coincided with a reduction in neuronal apoptosis and in brain infarction volume [37]. Exercise preconditioning induced neuroprotection after stroke, mediated by HSP-70 upregulation. Transgenic mice overexpressing HSP-27 and subjected to cerebral ischemia demonstrated neuroprotective benefits due to this overexpression [38]. Several clinical studies support the importance of regular physical exercise in stroke prevention [39, 40]. The mechanism of this beneficial effect is unknown, but HSPs may explain part of it.

**HSPs in Epilepsy**

HSP-27 has been found to be highly expressed in epileptic neocortex obtained from patients during neurosurgery, being present in astrocytes and in cerebral blood vessel walls [41]. Only low amounts of HSP-27 were detectable in control brains. This indicates that HSP-27 becomes induced in response to epileptic pathology. Although the functional aspects of such HSP-27 induction have yet to be elucidated, the authors claim that HSP-27 is a marker of cortical regions where seizures have caused a stress response. HSPBAP1, a protein inhibiting the function of HSP-27, is abnormally expressed in the neocortex of patients with intractable epilepsy, which may indicate a protective role for HSP-27 in epilepsy [42].

In brain tissue sections from epileptic patients, the HSP α,β-crystallin was overexpressed in astrocytes and oligodendrocytes, including satellite cells adherent to neurons, and occasionally in neurons of neocortex, hippocampus and amygdala. In some cases, the expression was most intense at or near the epileptic focus, with a diminishing gradient of intensity for 2–3 cm. α,β-Crystallin could therefore be a reliable tissue marker of epileptic foci [43].

**HSPs in Neurodegenerative Disorders**

In a recent study [44], HSPs were found to be upregulated in the hippocampus, inferior parietal lobe and cerebellum in subjects with mild cognitive impairment. The authors suggested that alteration in the chaperone protein function contributed to the pathogenesis and progression of Alzheimer’s disease. If true, targeting HSPs could be a therapeutic approach to delay the progression of the disease.

Protein aggregation with amyloid fibril formation in neurons is implicated in several neurodegenerative diseases [45]. The accumulation of α-synuclein in Lewy bodies plays a central role in the pathogenesis of Parkinson’s disease [46, 47]. In such patients, neurons positive for the HSP α,β-crystallin were found throughout the cerebral cortex, amygdala, and ventral claustrum. These neurons did not develop Lewy bodies, and neurons containing Lewy bodies did not accumulate α,β-crystallin [48], indicating a protective role for HSP α,β-crystallin in Parkinson’s diseases. This role remains to be confirmed by other studies.

Familial amyotrophic lateral sclerosis can be caused by mutations in Cu,Zn-superoxide dismutase-1 (SOD-1). In a recent study using a cell culture model, it was demonstrated that α,β-crystallin is capable of reducing aggregation of mutant SOD-1. This finding has implications regarding the role of chaperones in modulating the tissue-specific accumulations of SOD-1 in familial amyotrophic lateral sclerosis [49].

**Disclosure Statement**

The authors have no conflicts of interest to disclose.

**References**


