Heat-Shock Proteins in Clinical Neurology

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Key Words

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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 α , β -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 α , β -crystallin is expressed in epileptic foci. In neurodegenerative disorders such as Alzheimer dementia and Parkinson's disease, HSPs are upregulated in brain tissue, and α , β -crystallin modulates superoxide dismutase-1 (SOD-1) tissue accumulation in familial amy-

trophic 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.

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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 [28], 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 di-

minishing 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

- 1 Multhoff G: Heat-shock protein 70 (Hsp70): membrane location, export and immunological relevance. *Methods* 2007;43:229–237.
- 2 Bukau B, Horwich AL: The Hsp70 and Hsp60 chaperone machines. *Cell* 1998;92:351–366.
- 3 Tsan MF, Gao B: Heat-shock protein and innate immunity. *Cell Mol Immunol* 2004;1:274–279.

- 4 Li Z, Menoret A, Srivastava P: Roles of heat-shock proteins in antigen presentation and cross-presentation. *Curr Opin Immunol* 2002;14:45–51.
- 5 Wang Y, Kelly CG, Singh M, McGowan EG, Carrara AS, Bergmeier LA, Lehner T: Stimulation of Th1-polarizing cytokines, C-C chemokines, maturation of dendritic cells, and adjuvant function by the peptide binding fragment of heat-shock protein 70. *J Immunol* 2002;169:2422–2429.
- 6 Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK: HSP70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. *Nat Med* 2000;6:435–442.
- 7 Tebo AE, Szankasi P, Hillman TA, Litwin CM, Hill HR: Antibody reactivity to heat-shock protein 70 and inner ear-specific proteins in patients with idiopathic sensorineural hearing loss. *Clin Exp Immunol* 2006;146:427–432.
- 8 Tasneem S, Islam N, Ali R: Cross-reactivity of SLE autoantibodies with 70 kDa heat-shock proteins of *Mycobacterium tuberculosis*. *Microbiol Immunol* 2001;45:841–846.
- 9 Zlacka D, Vavrinova P, Hien Nguyen TT, Hromadnikova I: Frequency of anti-Hsp-60, -65 and -70 antibodies in sera of patients with juvenile idiopathic arthritis. *J Autoimmun* 2006;27:81–88.
- 10 Yusuf N, Nasti TH, Huang CM, Huber BS, Jaleel T, Lin HY, Xu H, Elmetts CA: Heat-shock proteins HSP27 and HSP70 are present in the skin and are important mediators of allergic contact hypersensitivity. *J Immunol* 2009;182:675–683.
- 11 Birtas-Atesoglu E, Inanc N, Yavuz S, Ergun T, Direskeneli H: Serum levels of free heat-shock protein 70 and anti-HSP70 are elevated in Behçet's disease. *Clin Exp Rheumatol* 2008;26:S96–S98.
- 12 Ratanachaiyavong S, Demaine AG, Campbell RD, McGregor AM: Heat-shock protein 70 (HSP70) and complement C4 genotypes in patients with hyperthyroid Graves' disease. *Clin Exp Immunol* 1991;84:48–52.
- 13 Hunt PJ, Marshall SE, Weetman AP, Bunce M, Bell JI, Wass JA, Welsh KI: Histocompatibility leucocyte antigens and closely linked immunomodulatory genes in autoimmune thyroid disease. *Clin Endocrinol (Oxf)* 2001;55:491–499.
- 14 Zhang ZY, Zhang Z, Schluesener HJ: Toll-like receptor-2, CD14 and heat-shock protein 70 in inflammatory lesions of rat experimental autoimmune neuritis. *Neuroscience* 2009;159:136–142.
- 15 Millar DG, Garza KM, Odermatt B, Elford AR, Ono N, Li Z, Ohashi PS: Hsp70 promotes antigen-presenting cell function and converts T-cell tolerance to autoimmunity in vivo. *Nat Med* 2003;9:1469–1476.
- 16 Stetler RA, Gao Y, Signore AP, Cao G, Chen J: HSP27: mechanisms of cellular protection against neuronal injury. *Curr Mol Med* 2009;9:863–872.
- 17 Lindström JM, Seybold ME, Lennon VA, Whittingham S, Duane DD: Antibody to acetylcholine receptor in myasthenia gravis: prevalence, clinical correlates, and diagnostic value. 1975 classical article. *Neurology* 1998;51:933–939.
- 18 Luchanok U, Kaminski HJ: Ocular myasthenia: diagnostic and treatment recommendations and the evidence base. *Curr Opin Neurol* 2008;21:8–15.
- 19 Evoli A, Batocchi AP, Minisci C, Di Schino C, Tonali P: Therapeutic options in ocular myasthenia gravis. *Neuromuscul Disord* 2001;11:208–216.
- 20 Wilcox N: Myasthenia gravis. *Curr Opin Immunol* 1993;5:910–917.
- 21 Li Z, Forester N, Vincent A: Modulation of acetylcholine receptor function in TE671 (rhabdomyosarcoma) cells by non-AchR ligands: possible relevance to seronegative myasthenia gravis. *J Neuroimmunol* 1996;64:179–183.
- 22 Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A: Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med* 2001;7:365–368.
- 23 McConville J, Farrugia ME, Beeson D, Kishore U, Metcalfe R, Newsom-Davis J, Vincent A: Detection and characterization of MuSK antibodies in seronegative myasthenia gravis. *Ann Neurol* 2004;55:580–584.
- 24 Fuhrer C, Sugiyama JE, Taylor RG, Hall ZW: Association of muscle-specific kinase MuSK with the acetylcholine receptor in mammalian muscle. *EMBO J* 1997;16:4951–4960.
- 25 Borges LS, Ferns M: Agrin-induced phosphorylation of the acetylcholine receptor regulates cytoskeletal anchoring and clustering. *J Cell Biol* 2001;153:1–11.
- 26 Romi F, Skeie GO, Gilhus NE, Aarli JA: Striation antibodies in myasthenia gravis: reactivity and possible clinical significance. *Arch Neurol* 2005;62:442–446.
- 27 Munakata S, Chen M, Aosai F, Kawaguchi N, Nemoto Y, Norose K, Hattori T, Yano A: The clinical significance of anti-heat-shock cognate protein 71 antibody in myasthenia gravis. *J Clin Neurosci* 2008;15:158–165.
- 28 Helgeland G, Petzold A, Hoff JM, Gilhus NE, Plant GT, Romi FR: Anti-heat-shock protein 70 antibody levels are increased in myasthenia gravis and Guillain-Barré syndrome. *J Neuroimmunol* 2010;225:180–183.
- 29 Yonekura K, Yokota S, Tanaka S, Kubota H, Fujii N, Matsumoto H, Chiba S: Prevalence of anti-heat-shock protein antibodies in cerebrospinal fluids of patients with Guillain-Barré syndrome. *J Neuroimmunol* 2004;156:204–209.
- 30 Salvetti M, Ristori G, Buttinelli C, Fiori P, Falcone M, Britton W, Adams E, Paone G, Grasso MG, Pozzilli C: The immune response to mycobacterial 70-kDa heat-shock proteins frequently involves autoreactive T cells and is quantitatively dysregulated in multiple sclerosis. *J Neuroimmunol* 1996;65:143–153.
- 31 Ce P, Erkizan O, Gedizlioglu M: Elevated HSP27 levels during attacks in patients with multiple sclerosis. *Acta Neurol Scand* 2011 (Epub ahead of print).
- 32 Stoevring B, Vang O, Christiansen M: α , β -crystallin in cerebrospinal fluid of patients with multiple sclerosis. *Clin Chim Acta* 2005;356:95–101.
- 33 Van Noort JM, Verbeek R, Meilof JF, Polman CH, Amor S: Autoantibodies against α , β -crystallin, a candidate autoantigen in multiple sclerosis, are part of a normal human immune repertoire. *Mult Scler* 2006;12:287–293.
- 34 Sinclair C, Mirakhur M, Kirk J, Farrell M, McQuaid S: Up-regulation of osteopontin and α , β -crystallin in the normal-appearing white matter of multiple sclerosis: an immunohistochemical study utilizing tissue microarrays. *Neuropathol Appl Neurobiol* 2005;31:292–303.
- 35 Steinman L: A molecular trio in relapse and remission in multiple sclerosis. *Nat Rev Immunol* 2009;9:440–447.
- 36 Azarpazhooh MR, Mobarra N, Parizadeh SM, Tavallaie S, Bagheri M, Rahsepar AA, Ghayour-Mobarhan M, Sahebkar A, Ferns GA: Serum high-sensitivity C-reactive protein and heat-shock protein 27 antibody titers in patients with stroke and 6-month prognosis. *Angiology* 2010;61:607–612.
- 37 Liebelt B, Papapetrou P, Ali A, Guo M, Ji X, Peng C, Rogers R, Curry A, Jimenez D, Ding Y: Exercise preconditioning reduces neuronal apoptosis in stroke by up-regulating heat-shock protein-70 (heat-shock protein-72) and extracellular-signal-regulated-kinase 1/2. *Neuroscience* 2010;166:1091–1100.
- 38 Van der Weerd L, Tariq Akbar M, Aron Bardin R, Valentim LM, Thomas DL, Wells DJ, Latchman DS, Gadian DG, Lythgoe MF, de Belleruche JS: Overexpression of heat-shock protein 27 reduces cortical damage after cerebral ischemia. *J Cereb Blood Flow Metab* 2010;30:849–856.
- 39 Goldstein LB: Physical activity and the risk of stroke. *Expert Rev Neurother* 2010;10:1263–1265.
- 40 Zhang F, Wu Y, Jia J: Exercise preconditioning and brain ischemic tolerance. *Neuroscience* 2011;177:170–176.
- 41 Bidmon HJ, Görg B, Palomero-Gallagher N, Behne F, Lahl R, Pannek HW, Speckmann EJ, Zilles K: Heat-shock protein-27 is upregulated in the temporal cortex of patients with epilepsy. *Epilepsia* 2004;45:1549–1559.

- 42 Xi ZQ, Sun JJ, Wang XF, Li MW, Liu XZ, Wang LY, Zhu X, Xiao F, Li JM, Gong Y, Guan LF: HSPBAP1 is found extensively in the anterior temporal neocortex of patients with intractable epilepsy. *Synapse* 2007;61: 741–747.
- 43 Sarnat HB, Flores-Sarnat L: α , β -crystallin as a tissue marker of epileptic foci in paediatric resections. *Can J Neurol Sci* 2009;36:566–574.
- 44 Di Domenico F, Sultana R, Tiu GF, Scheff NN, Perluigi M, Cini C, Butterfield DA: Protein levels of heat-shock proteins 27, 32, 60, 70, 90 and thioredoxin-1 in amnesic mild cognitive impairment: an investigation on the role of cellular stress response in the progression of Alzheimer disease. *Brain Res* 2010;1333:72–81.
- 45 Ecroyd H, Carver JA: The effect of small molecules in modulating the chaperone activity of α , β -crystallin against ordered and disordered protein aggregation. *FEBS J* 2008;275: 935–947.
- 46 Khandelwal PJ, Dumanis SB, Feng LR, Maguire-Zeiss K, Rebeck G, Lashuel HA, Mousa CE: Parkinson-related parkin reduces α -synuclein phosphorylation in a gene transfer model. *Mol Neurodegener* 2010;5:47.
- 47 Grünewald A, Voges L, Rakovic A, Kasten M, Vandebona H, Hemmelmann C, Lohmann K, Orolicki S, Ramirez A, Schapira AH, Pramstaller PP, Sue CM, Klein C: Mutant parkin impairs mitochondrial function and morphology in human fibroblasts. *PLoS One* 2010;5:e12962.
- 48 Braak H, Del Tredici K, Sandmann-Kiel D, Rüb U, Schultz C: Nerve cells expressing heat-shock proteins in Parkinson's disease. *Acta Neuropathol* 2001;102:449–454.
- 49 Karch CM, Borchelt DR: An examination of α , β -crystallin as a modifier of SOD-1 aggregate pathology and toxicity in models of familial amyotrophic lateral sclerosis. *J Neurochem* 2010;113:1092–1100.