Pharmacological Modulation of the Heat Shock Response

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Abstract Life presents a continuous series of stresses. Increasing the adaptation capacity of the organism is a long-term survival factor of various organisms and has become an attractive field of intensive therapeutic research. Induction of the heat shock response promotes survival after a wide variety of environmental stresses. Preclinical studies have proven that physiological and pharmacological chaperone inducers and co-inducers are an efficient therapeutic approach in different acute and chronic diseases. In this chapter, we summarize current knowledge of the current state of chaperone modulation and give a comprehensive list of the main drug candidates.

1
Stress and the Heat Shock Response

Life is stress, a continuous challenge from molecules to mind. Sophisticated and robust protective mechanisms have been and are still evolving to promote survival under constantly changing environmental conditions. One of the most ancient and highly conserved adaptive mechanisms in the cellular setting is the heat shock or stress response. Molecular chaperones or heat shock proteins maintain the conformational homeostasis of the proteome (Thirumalai and Lorimer 2001; Young et al. 2004). Most chaperones are essential proteins present at high concentrations even under nonstress conditions, emphasizing their vital housekeeping role. However, many types of toxic insults (e.g., heat shock, ethanol, oxidative stress) lead to a sudden rise in chaperone levels. Chaperone induction is mediated at the transcriptional level by an autoregulatory feedback loop. An increase in misfolded proteins results in the release of heat shock transcription factor 1 (HSF-1) from the repressing Hsp90/Hsp70/Hsp40 complex and a subsequent activation of heat shock gene transcription (see the chapter by R. Voellmy, this volume).

2
Chaperone-Mediated Cytoprotection

A sublethal stress exposure protects the cell from the deleterious effect of a subsequent otherwise lethal stress. The stress tolerance (thermotolerance in case of the prototype, heat shock) is mediated by the elevated levels of chaperones, especially Hsp70, Hsp27, and Hsp90 (Welch 1992). Chaperones play a crucial role in such vital processes as signal transduction, transport processes, cell division, migration, and differentiation, and they are indispensable for proper immune function. Moreover, from a general point of view, chaperones are stabilizing hubs of the cellular protein–protein/lipid networks (reviewed in Csermely 2004, 2005; Sőti et al. 2005).

The cytoprotective role of chaperones involves a direct stabilization of macromolecular structure of proteins and lipids (Török et al. 2001; Tsvetkova et al. 2002). Moreover, Hsp70 and Hsp110 stabilize mRNA structure (Henics et al. 1999), and cytokine mRNAs are stabilized through Hsp70 induction or proteasome downregulation (Laroia et al. 1999). Intriguingly, chaperones are critical factors in apoptosis. They play both a direct role by supporting key molecules in (anti)apoptotic signaling (APAF-1, Bcl-2), and an indirect role by being involved in antioxidative defense and scaffolding macromolecular assemblies (Sreedhar and Csermely 2004). Various studies have demonstrated that chaperone-mediated cytoprotection can be largely attributed to the suppression of apoptosis. Cells failing to mount a stress response are sensitive to apoptosis (Sreedhar et al. 1999). ATP depletion is hallmark of many disease states, including a variety of ischemic conditions. It results in a rapid metabolic incompetence accompanied by profound cell death. Both stress preconditioning and transient Hsp70 overexpression in rat cardiac myoblasts were shown to exert a marked reduction in cell death as well as in the amount of total denatured protein (Kabakov et al. 2002). In separate studies, hypoxia/reoxygenation-induced cell death was rescued by a preceding heat shock in rat cardiac myocytes (Tanonaka et al. 2003). Heat shock attenuated poly(ADP-ribose) polymerase (PARP) activation, and pharmacological PARP inhibition prevented the cell death evoked by the treatment. Interestingly, Hsp70 expression and nuclear translocation were observed upon heat shock, raising the possibility of an inhibitory Hsp70–PARP interaction during heat shock/hypoxia. Moreover, Hsp70 contributes to the increased antioxidative defense during ischemia/reperfusion (Chong et al. 1998).

Accumulation of mutant, misfolded proteins is a threat for postmitotic cells, especially for the nervous system. Protein aggregation is a complex process, harboring a wide variety of cytotoxic events, inducing cell death (Stefani and Dobson 2003; Bossy-Wetzel et al. 2004). Chaperones were shown to be associated both with oligomeric and aggregated species and protected from cell death in *Drosophila*, in mammalian cells, and in transgenic mice (Warrick et al. 1999; Carmichael et al. 2000; Cummings et al. 2001).

Intriguingly, not only cytosolic chaperones are able to confer cytoprotection. Endoplasmic reticulum chaperones, normally induced by the unfolded protein response, are also upregulated upon excitotoxic and oxidative insults and display protective effect via diminishing the production of reactive oxygen species and stabilizing calcium homeostasis (Yu et al. 1999). Similarly, upregulation of endoplasmic reticulum chaperones in epithelial cells was shown to exert a robust effect against ATP-depletion-induced cell damage (Bush et al. 1999).

3
Possible Therapeutic Use of Chaperone Induction

Since chaperones protect the cells from a wide variety of physiological and pathological of stressors, virtually any conditions associated with (a) increased cellular/organismal stress and/or (b) decreased protective potential may be therapeutic target (Table 1). The first category encompasses two subgroups: the first with increased oxidative stress/ATP depletion, the prototype is the ischemic diseases; the second is the direct toxicity of aberrant proteins, including the progressive neurodegenerative diseases. The remainder of the conditions is best represented by aging, where the stress continuously uses up the different adaptation mechanisms, including a proper mounting of the heat shock response, resulting in a vicious circle and finally exhaustion.

Table 1 Pathological states as possible therapeutic targets for chaperone upregulation[a]

Pathology	Reference
Increased stress:	Christians et al. 2002
Ischemia-reperfusion	
Cardiovascular disease	Snoeckx et al. 2001
Stroke	DeFranco et al. 2004
Inflammation, shock	van Molle et al. 2002
Toxin exposure	
Ethanol	?
Drugs	?
Oxidants	Papp et al. 2003
Trauma and regeneration	Kalmar et al. 2002, 2003
Transplantation	Perdrizet et al. 1993
Low-frequency electromagnetic field	Goodman and Blank 2002
Proteinopathies	Welch 2004
Amyloidosis and neurodegeneration	Stefani and Dobson 2003
Cystic fibrosis (and others)	Amaral 2004
Diabetes mellitus	Nánási and Jednákovits 2001
Mixed, with decreased protection	
Aging	Sőti and Csermely 2003; Hsu et al. 2003
Hyperlipidemia	Csont et al. 2002
Overnutrition	?
Recurrent (viral) infections	Rosi et al. 1996
Chronic fatigue syndrome	?

[a]Question marks indicate lack of experimental evidence

3.1
Ischemia Reperfusion

Ischemic heart disease is one of the most intensely studied diseases with respect to chaperone protection. Besides their anti-apoptotic role, chaperones contribute to proper functioning by the sustained activation of endothelial nitric oxide synthase (eNOS) provided by Hsp90 (Kupatt et al. 2004), the maintenance of redox homeostasis (Chong et al. 1998; Papp et al. 2003), and the enhancement of mitochondrial respiratory complex activity (Sammut and Harrison 2003). Female heart contains twice as much inducible Hsp70 than male heart, due to the presence of estradiol (Vos et al. 2003), which induces HSF-1 transactivation (Knowlton and Sun 2001). Both the HSF-1-inducing action and cardioprotective effect of estrogen is mediated by NFκB activation (Hamilton

et al. 2004). Ovariectomy reduces and estrogen replacement re-establishes cardiac Hsp70 expression (Vos et al. 2003), supporting the cardioprotective role of estrogen replacement therapy after menopause, and raising the question of administering estrogen agonists to patients with high cardiac risks. On the other hand, exercise preferentially induced cardiac Hsp70 and exerted cardioprotection only in male as well as ovariectomized female rats, suggesting that training may be much more important for males than for females in defending against the effects of heart disease and offers a novel manner by which males may reduce the sex gap in susceptibility to adverse cardiac events (Paroo et al. 2002). However, cardiac function and postischemic adaptation is deteriorated already in middle-aged rats, despite a relatively maintained chaperone inducibility (Honma et al. 2003), arguing against considering chaperone induction as a miraculous remedy. The central importance of a healthy diet is emphasized by the finding that experimental hyperlipidemia attenuated the heat shock response in rat hearts (Csont et al. 2002). Further research will reveal the proper place of chaperone-inducing therapies in cardiovascular diseases.

There are several examples suggesting that chaperones play an important role in protecting the nervous system both in acute ischemic conditions such as stroke (DeFranco et al. 2004) and chronic degenerative states such as vascular dementia and neuronal proteinopathies such as Alzheimer's, Parkinson's, and other diseases (Stefani and Dobson 2003). Interestingly, glial Hsp70 is released and taken up by nerve cells and enhances neuronal stress tolerance (Guzhova et al. 2001). Another intriguing finding is that chaperone induction by the amino acid analog canavanine attenuated the retinopathic complication of streptozotocin-induced diabetes in rats, prompting further investigations of chaperone inducers in easing the chronic consequences of diabetes such as retinopathy (Mihály et al. 1998).

3.2
Inflammation and Sepsis

Inflammation poses a stress on immune cells. Indeed, Hsp70 is overexpressed in polymorphonuclear cells under sepsis (Hashiguchi et al. 2001), and Hsp70 conferred an augmented antioxidative response and inhibited apoptosis (Hashiguchi et al. 2001). Hsp70 overexpression also conferred cytoprotection by inhibiting NFκB activation and iNOS upregulation in rats, ameliorating cardiac shock and hypotension upon endotoxic shock (Hauser et al. 1996; Chan et al. 2004). Another protective feature of Hsp70 overexpression is that it prevented high production of LPS-induced inflammatory cytokines in human macrophages (Ding et al. 2001). Similarly, heat shock inhibited IL-6 and iNOS expression upon TNFα treatment in wild type, but not in heat-inducible hsp70.1 gene-deficient mice (van Molle et al. 2002). Heat shock reduced bowel damage and apoptosis, circumventing the severe side effects of antitumor protocols based on TNF and interferon-gamma, leading to a significant inhibition

of lethality but not to a reduction of antitumoral capacity (van Molle et al. 2002).

3.3
Aging and Chaperone Overload

The positive correlation between longevity and a robust heat shock response is an experiential fact and is well documented in several studies using *Caenorhabditis elegans* as a model system, implicating the chaperone network as one of the critical adaptive mechanisms (Garigan et al. 2002; Hsu et al. 2003). Chaperone inducibility generally decreases during aging. Hsp70 overdose, under the control of its own promoter, extended the lifespan in *Drosophila*. Many experimental manipulations inducing life-span extension also upregulate chaperones and lead to a stress response of higher intensity (Sőti and Csermely 2003). Chaperones may be overburdened by the individual life in aging, as well as during the improvement of living standards over the last two centuries. This phenomenon, the so-called chaperone overload, may be a causative factor in a number of degenerative diseases and aging (Csermely 2001; Sőti and Csermely 2003). Whether strengthening the heat shock response would further aggravate or solve this problem may be a key question in the coming years of research.

The above-mentioned examples show the power of a properly mounted stress response in different pathological conditions.

4
Heat Shock Response Modulators

The heat shock or stress response is a multistep process involving cross-talk among different processes such as growth and stress-activated signaling pathways, protein misfolding, aggregation and degradation, heat shock, and other transcription factor activation, with a subsequent production of heat shock genes. For the sake of simplicity and specificity, we will focus on the inhibitors of misfolded protein degradation and activators of Hsp synthesis as candidates for therapeutic intervention.

Ancient cultures understood that sauna is a well known practice in a healthy lifestyle. Heat shock is the archetype of preconditioning, and was already extensively discussed in the literature. Mild heat stress leads to a series of beneficial effects in cells (Park et al. 2005) and hormesis induced by repeated mild heat shock is a promising means to preserve the adaptation capacity of cells (Rattan 2004). In this section, we will focus on different approaches to induce the heat shock response.

4.1
Aspirin as a Chaperone Co-inducer

The first pharmacological agents shown to affect the stress response were sodium salicylate and aspirin (Jurivich et al. 1992). Salicylate induced HSF-1 DNA binding, did not lead to hsp transcription per se, but augmented the stress response upon a challenging insult. Unfortunately, these compounds are not specific, and besides the inhibition of cyclo-oxygenase, both compounds bind to Grp78 (BiP) and inhibit its ATPase activity and may interfere with its activity (Deng et al. 2001).

4.2
Glutamine Is a Remedy for the Critically Ill

Several physiologically occurring compounds activate chaperone expression. The amino acid glutamine, an important nutrient for bowel and muscle cells, is a very potent inducer of chaperone expression in *Drosophila* cells as well as in human patients (Sanders and Kon 1991; Wischmeyer 2002, 2004). It shows all the beneficial actions of heat stress, including a marked improvement in survival during sepsis, antiapoptotic and anti-inflammatory properties, and it depends on the induction of Hsp70. Even after a single glutamine injection, Hsp70 induction can be observed in the gut, blood cells, lung and heart, among other organs, suggesting a fairly general phenomenon. For instance, glutamine administration before cardiopulmonary bypass reduced the inflammation and improved clinical outcome in rats (Hayashi et al. 2002). Though the mechanism of action of glutamine is not known, it is devoid of any toxicity and is already widely used in enteral and parenteral nutrition, by heavy exercise athletes, and by naturopathic doctors for bowel problems. It must be noted that during prolonged exercise and sepsis or major trauma, the blood glutamine level is decreased, and such glutamine depletion impairs the stress response, probably contributing to susceptibility to exhaustion, and worsening (Oehler et al. 2002). There is ample opportunity for clinical use in critically ill patients with major trauma, surgery, or sepsis.

4.3
Zinc Supplementation Is a Prerequisite of Proper Chaperone Induction

Zinc is an important trace element that supports the function of several enzymes, including antioxidant enzymes and transcription factors with zinc finger motifs, such as steroid receptors. Zinc is critical for proper immune function, and it was shown that a 3-week dietary zinc depletion reduces Hsc70, Hsp40, and Hsp60 expression to approximately 50% in murine thymus (Moore et al. 2003). The same study showed that zinc overdose led to a reduction in heat shock mRNA levels, highlighting the importance of proper zinc consumption and supplementation. Both zinc bioavailability and immune function is

decreased in the aged population. On the contrary, both are better preserved in the successfully aged centenarians. While in vitro zinc supplementation somewhat augmented the heat-induced Hsp70 expression in lymphoblasts from aged human donors, it diminished it both in young and centenarian samples (Ambra et al. 2004). This finding may be explained by the hypothesis that chaperones may regulate metallothionein metabolism (Mocchegiani et al. 2000). Zinc induces a heat shock response in cell culture (Hatayama et al. 1993), as well as in gastric mucosal and hepatic cells in vivo (Odashima et al. 2002; Cheng et al. 2002). Besides the general protective effect of zinc, it can be used before major surgery, or even before transplantation for organ preservation (Cheng et al. 2002), since it is well known that chaperones are helpful in graft preservation (Perdrizet et al. 1993).

4.4
Hsp90 Inhibitors Are Useful Cytoprotective Agents

Hsp90 inhibitors are multitarget antitumor drugs: by targeting the ATP-binding site of Hsp90 they compromise several growth and survival pathways in parallel (Neckers 2003; see also the chapter by L. Neckers, this volume). Clinical implications of geldanamycin, radicicol, and other Hsp90 inhibitors are summarized in the chapter by S. Pacey et al. in this volume. It seems to be reasonable to assume that heat shock protein inhibitors may lead a compensatory stress response. Indeed, at a very low dose where cytotoxic effects cannot be observed, geldanamycin releases HSF-1 from Hsp90 and leads to heat shock protein expression. This phenomenon was already successfully used in different experimental models to induce cytoprotection. Geldanamycin treatment improved Parkinsonism in *Drosophila* (Auluck and Bonini 2002), and both geldanamycin and radicicol were effective in cellular and mouse models of polyglutamine diseases, actually better than overexpression of Hsp70 (Sittler et al. 2001; Hay et al. 2004). Moreover, geldanamycin also binds to Grp94, the ER-resident Hsp90, and induces cytoprotective ER chaperones via the unfolded protein response (Lawson et al. 1998), which widens the therapeutic potential of these drugs. Radicicol, representing another class of Hsp90 inhibitors, exerted a cardioprotective effect in ischemia/reperfusion injury (Griffin et al. 2004). Herbimycin A, a geldanamycin-related compound and a Tyr-phosphatase inhibitor, was also able to reduce intimal hyperplasia (restenosis) by upregulating Hsp27 after balloon angioplasty (Conolly et al. 2003).

4.5
Proteasome Inhibition Activates Cytoprotection

As HSF-1 is activated by an increased flux of misfolded proteins, proteasome inhibitors are also potent inducers of both the cytosolic and the ER chaperones and thermotolerance (Bush et al. 1997). MG-132 and lactacystin in-

creased IL-6 production by human intestinal epithelial cells (Pritts et al. 2002), while MG-132 and MG-262 treatment resulted in better contractile function and faster recovery of heart papillary muscle after ischemia (Stangl et al. 2002). It must be mentioned, though, that the protective response evoked by proteasome inhibitors is not merely based on chaperone induction. First, activation of heat shock elements is not sufficient to induce αB-crystallin expression upon proteasome inhibition (Aki et al. 2003). Second, in some model systems these compounds inhibit HSF-1 dephosphorylation and transactivation if they are present after heat shock (Kim and Li 1999). Third, a low dose of MG-132 and lactacystin protects neural cells from excitotoxic and oxidative stress without chaperone induction (Lee et al. 2004). Thus, further studies are needed to clarify the cytoprotective mechanisms of proteasome inhibitors.

4.6
Anti-ulcer Drugs Induce Chaperones

Other heat shock protein inducers are emerging as promising tools in a wide variety of pathophysiological states. Geranylgeranylacetone is nontoxic anti-ulcer drug, and recently has been shown to induce the heat shock response. This property makes it a useful intervention in dysfunctions of the stomach and probably the digestive tract (Rokutan 2000). Its use is also proven in hepatocellular damage originating either from ethanol- or oxidant-related pathology (Ikeyama et al. 2001), or from hepatectomy (Oda et al. 2002). Systemic geranylgeranylacetone treatment protected retinal ganglion cells in a rat glaucoma model (Ishii et al. 2003) and hippocampal neurons against ischemia (Fujiki et al. 2004); however, in addition to chaperones, other protective mechanisms may be involved. Carbenoxolone is another anti-ulcer drug that possesses Hsp70-inducing activity (Nagayama et al. 2001). However, we lack further studies to prove its therapeutic benefit.

4.7
Prescription Drugs May Induce a Heat Shock Response

Several medicines were studied with respect to cytoprotective action. Estrogen was found to affect the inducibility of chaperones. Other steroids such as the glucocorticoids, hydrocortisone, or the agonists methylprednisolone and dexamethasone were shown to elicit a cardioprotective effect by inducing Hsp70, transcriptionally and/or post-transcriptionally (Sun et al. 2000; Valen et al. 2000). Whether other hormones behave the same way is an open question.

Cyclosporine A (CsA) is a toxic immunosuppressive drug. On the one hand, both heat shock and CsA preconditioning produce tolerance against the CsA-toxicity (Yuan et al. 1996). On the other hand, CsA is an activator of HSF-1 and -2, probably an inhibitor of the proteasome, but leads only to Hsp27

upregulation (Paslaru et al. 2000). These findings raise the possibility that toxic side effects of CsA may be circumvented by a low-dose administration preceding the therapeutic dose.

Surprisingly, the commonly used antibiotics ampicillin and ceftriaxone—previously thought to be specifically damaging to bacteria—upregulate Hsp27 and Hsp60 in human lymphocytes and protect them from staurosporine-induced apoptosis (Romano et al. 2004). The generality of this phenomenon with respect to cell types, antibiotics, and other harmful insults demands a systematic study. On the other hand, it should be noted that many cell culture facilities routinely use antibiotics, especially ampicillin, and this may produce misleading results in apoptosis and chaperone induction assays.

4.8
Herbal Medicines Contain Potent Cytoprotective Compounds

Traditional medicine has used herbal compounds for thousands of years for pain relief, fever reduction, infection and inflammation, stimulation of physical production, and for treating tumors. Among others, celastrols have recently been documented as cytoprotective agents and heat shock response inducers (Westerheide et al. 2004). The naturally occurring antioxidant ergothioneine augmented Hsp70 induction and conferred protection against liver ischemia/reperfusion in the rat (Bedirli et al. 2004).

Curcumin is dietary pigment of turmeric, a favorite spice in the culinary arts. It is also a remedy in Indian medicine. Curcumin increased Hsp70 levels and had a cytoprotective effect (Sood et al. 2001). It was harmless in normal cells; however, it induced a pronounced apoptosis in different tumor cell lines (Khar et al. 2001). Intriguingly, resistant lines had a robust heat shock response upon curcumin treatment (Khar et al. 2001), suggesting that among the pleiotropic effects of curcumin, the accentuated heat shock response may be critical for tumor survival. This finding raises two ideas. First, curcumin may be effectively combined with heat shock protein inhibitors in antitumor protocols to elicit maximal beneficial potential. Second, curcumin is a unique drug that kills tumor cells, while strengthening normal cells, which may make it an ideal panacea for cancer patients.

4.9
Cyclopentenone Prostaglandins: Antiviral Drug Candidates

Naturally occurring anti-inflammatory cyclopentenone prostaglandins also induce Hsp70. These molecules are characterized by the presence of a reactive α,β-unsaturated carbonyl group in the cyclopentane ring (cyclopentenone), which is the key structure for triggering HSF-1 activation. 2-Cyclopenten-1-one selectively induces Hsp70 in human cells, and this is associated with antiviral activity (Rossi et al. 1996). These prostaglandin derivatives also dis-

play an HSF-1/Hsp70-dependent anti-inflammatory action (Ianaro et al. 2003), which makes them promising antiviral drug candidates. However, many of the effects are mediated via the NFκB pathway, given that these compounds are direct inhibitors of the IκB kinase (Rosi et al. 2000). They may be very attractive compounds in reducing viral infection and overwhelming inflammatory reactions.

4.10
Nutrition State Influences Hsp Response

Though not pharmacological compounds, some effects from the environment also have the potential to regulate the heat shock response. Calorie restriction retards aging and oxidative stress, and induces heat shock proteins (Sőti and Csermely 2003). Both calorie restriction and its mimetic, 2-deoxyglucose, reduce focal ischemic brain damage, and induce Hsp70 (Yu and Mattson 1999), which implies that a modest diet may protect from several age-related pathologies by strengthening the natural adaptation mechanisms, including the stress response.

4.11
Low-Frequency Electromagnetic Fields: Beneficial Potential and Health Hazard

Our environment is not merely material. There is a continuous spectrum of electromagnetic radiation surrounding and penetrating us. While higher energy UV and ionizing radiations are recognized as life-threatening and stress response-inducing stimuli, we have only just begun to explore the biological effects of lower-frequency fields. Two segments of low-frequency fields are especially interesting. The first is the radio-frequency (athermal) microwave field (in the MHz range) widely used in wireless communication, e.g., in cell phones, while the other is the extremely low-frequency field (ELF, below 300 Hz) present everywhere. While proper ELF exposure leads to a variety of regenerative processes, improper exposure to both fields are proven to pose a health hazard to humans. Interestingly, acute exposure to both athermal microwave radiation (915 MHz) and to ELF (60 Hz) induced a heat shock response and cytoprotection against hypoxia-reperfusion in chick embryos (Shallom et al. 2002). Indeed, it is well documented that there is an ELF responsive element in several genes, including Hsp70, which may directly sense the electromagnetic radiation (Goodman and Blank 2002).

There are several advantageous properties to ELF radiation and preconditioning:

– It is noninvasive, safe, comfortable, and simple to administer, even repeatedly before, during, and after cardiac surgery to mount a higher stress response.

- It is more effective in inducing Hsp70 than heat: the energy density required is 14 orders of magnitude lower for ELF than for heat (which may also mean a harmful effect at this low energy input!).

- ELF easily penetrates the body, allowing systemic administration, but even can be directed to the target region.

- The same ELF exposure induces Hsp70 in all systems from bacteria to human, emphasizing the presently unknown importance of this range (Goodman and Blank 2002).

Promising biomedical applications of ELF and chaperones are emerging. One opportunity is that ELF responsive elements can be used as novel, non-invasive techniques in transgene expression in a highly regulated fashion, a possible application in gene therapy with a sensory/ELF-generating circuit (e.g., a glucose sensor, an ELF generator, and cells containing the insulin gene with ELF element in its promoter; Goodman and Blank 2002). The second application is the preconditioning driven by ELF treatment, as mentioned above. The third is that chaperones may be much better markers of ELF exposure than the traditionally used heat absorption of tissues.

From a public health standpoint, it is important that more studies be performed to determine if repeated exposures, a condition likely to be found in the everyday setting (e.g., in cell phone use), are still beneficial. It is also important how our body is saturated with chronic ELF and whether a therapeutic ELF exposure would result in chaperone expression. One study showed that similarly to heat shock and other stresses, chronic ELF (only for 4 days) stimulation led to a distress: the exhaustion of the heat shock response and downregulation of Hsp70 with a consequent decline in cytoprotection (Di Carlo et al. 2002). This may explain epidemiologic correlations between chronic ELF exposure and cancer and may question the use of chaperone expression to determine the extent of chronic ELF exposure.

4.12
Chaperone Co-inducers: A Safer Opportunity to Induce the Heat Shock Response

The previous section highlighted that instead of the expected hormesis an overdose of chaperone inducers may lead to distress over the long term. On the contrary, a multicopy of inducible Hsp70 with its own promoter extended life in *Drosophila* (Tatar et al. 1997). Therefore drugs only augmenting the naturally occurring stress response (called chaperone co-inducers; Vígh et al. 1997) may be more beneficial without the side effects of chronic stress. Chaperone co-inducers are representatives of multitarget, low-affinity drugs, which may have a much better efficiency than single-target high-affinity drugs developed by rational drug design (Csermely et al. 2005). The lead compound, a nontoxic hydroxylamine derivative, Bimoclomol, helps the induction of Hsp synthesis

by both perturbing various membrane structures and helping the release of putative lipid-signaling molecules as well as by the prolongation of the binding of HSF-1 to the heat shock elements on the DNA (Hargitai et al. 2003; Török et al. 2003; Vígh et al. 1997). Bimoclomol binds to HSF-1 with a low affinity, which may contribute to its effect to prolong HSF-1 binding to DNA. Chaperone co-inducers also stabilize membranes, which may be of special importance to prevent apoptotic events related to the decomposition of cardiolipin and the consecutive destabilization of the mitochondrial membrane (Török et al. 2003).

Chaperone co-inducers lead to heat shock protein expression after stress, display strong cytoprotective effect (Vígh et al. 1997), and have great beneficial potential in a wide variety of pathological conditions. Bimoclomol was successfully used in cardiovascular and diabetic complications (Nánási and Jednákovits 2001). BRX-220, a potent analog of Bimoclomol, protected against acute pancreatitis, by diminishing serum markers and enhancing antioxidative capacity (Rakonczay et al. 2002a). However, it should be noted that this effect may not be solely due to Hsp induction, since arsenite treatment effectively inducing Hsp70 did sufficiently protect in this experimental pancreatitis model (Rakonczay et al. 2002b). Kürthy et al. (2002) showed a beneficial effect of BRX-220 against insulin resistance and peripheral neuropathy in diabetic rats. BRX-220 was a useful remedy in peripheral nerve injury (Kalmar et al. 2002, 2003). Recently arimoclomol was successfully applied in an inherited neurodegenerative disease, amyotrophic lateral sclerosis (ALS) (Kieran et al. 2004). ALS is characterized by a mutation in Cu/Zn superoxide dismutase-1, with loss of function (increased oxidative stress) and gain of function (protein aggregation) in spinal motoneurons. Arimoclomol delayed the onset of and led to a 22% life extension in amyotrophic lateral sclerosis in a mouse model (Kieran et al. 2004), suggesting that this class of drugs may prove to be effective in other neurodegenerative diseases.

5
Conclusions and Perspectives

Chaperone inducers are physiological compounds or potent drugs with pleiotropic beneficial actions. Instead of the old paradigm of meticulously targeting single molecules or eliminating disease-causing reasons, they focus on enhancing the natural protective capacity of our own body (Csermely et al. 2005). This protection is brought about by a higher level of heat shock or stress proteins and may provide an important novel therapeutic approach in a number of acute and chronic diseases and aging, even with a combination of traditional medications. Chaperone co-inducers may circumvent the distress-exhaustion cycle by overstimulation, since they only augment the naturally occurring Hsp induction. Further research will clarify the effect of long-term treatment and clinical applications, especially the relationship with chaperone overload.

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