Adaptogens exert a stress-protective effect by modulation of expression of molecular chaperones

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Abstract

Adaptogens are medicinal plants that augment resistance to stress, and increase concentration, performance and endurance during fatigue. Experiments were carried out with BALB/c mice taking ADAPT-232 forte, a fixed combination of three genuine (native) extracts of \textit{Eleutherococcus senticocus}, \textit{Schisandra chinensis} and \textit{Rhodiola rosea}, characterised for the content of active markers eleutherosides, schisandrin, salidroside, tyrosol and rosavin and in doses of about 30, 90 and 180 mg/kg for seven consecutive days followed by forced swimming test to exhaustion. ADAPT-232 forte strongly augments endurance of mice, increasing the time taken to exhaustion (TTE) in a dose-dependent manner from $3.0 \pm 0.5$ to $21.1 \pm 1.7$ min, approximately seven fold. Serum Hsp72 was measured by EIA both in normal and stressful conditions before and after swimming test. Repeated administration of adaptogen dose dependently increases basal level of Hsp72 in serum of mice from $0.8–1.5$ to $5.5–6.3$ pg/ml. This effect is even stronger than the effect of stress, including both physical (swimming) and emotional impacts: $3.2 \pm 1.2$ pg/ml. Cumulative effect of stress and adaptogen was clearly observed in groups of animals treated with adaptogen after swimming to exhaustion, when serum Hsp72 increased to $15.1 \pm 1$ pg/ml and remained at almost the same level during the 7 days. It can be concluded that adaptogens induce increase of serum Hsp72, regarded as a defense response to stress, and increase tolerance to stress (in our model combination of physical and emotional stresses). It can be suggested that increased tolerance to stress induced by adaptogen is associated with its stimulation of expression of Hsp70 and particularly with Hsp72 production and release into systemic circulation, which is known as a mediator of stress response involved in reparation of proteins during physical load. Our studies suggest that this could be one of the mechanisms of action of plant adaptogens.

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Introduction

Adaptogens are medicinal plants that enhance the “state of non-specific resistance” of an organism to stress, augmenting resistance to physical, biological, chemical and psychological stresses, and increasing concentration, performance and endurance during fatigue (Brekhman and Dardymov 1968; Panossian...
2003; Panossian and Wikman 2005; Panossian and Wagner 2005; Olson et al. 2008). The mechanism of action of adaptogens is partially associated with the hypothalamic–pituitary–adrenal (HPA) axis, a part of the stress system that also contributes to the nervous, cardiovascular, immune, gastrointestinal and endocrine systems (Panossian et al. 1999a). Involvement of cortisol, nitric oxide, stress-activated protein kinase JNK and forkhead box O transcription factor (DAF-16) in stress-protective effects of adaptogens was documented in several studies (Panossian et al. 1999b,c, 2007; Wiegant et al. 2008a,b); however a role of molecular chaperons (heat shock proteins) in adaptogen-induced resistance to stress has to be clarified (Ip et al. 2001; Chiu and Ko 2004; Wiegant et al. 2008a,b).

Molecular chaperons are a group of proteins that promote correct three-dimensional folding of other proteins, prevent their aggregation and assist in re-folding of misfolded proteins, which are the main contributors to many devastating human diseases. The 70-kDa heat shock protein Hsp72 plays a central role in the mechanism that rids the cell of stress-induced misfolding or incompletely synthesized polypeptides that otherwise would interfere with normal cellular function, thereby playing critical roles in maintaining cellular homeostasis and in protecting the cell from stressful conditions and increase cell survival in the face of otherwise lethal cellular stress. In addition, Hsp72 may function as an endogenous “danger signal” for the immune system. The danger theory postulates that immune activation involves danger/non-danger molecular recognition schemas and suggests that innate immune cells are activated by danger signals that are derived from stressed or damaged self-proteins (Matzinger 1998; Gallucci and Matzinger 2001). It is now widely accepted that circulating serum Hsp72 fits this criteria (Asea 2007, 2008). Exposure to physical or psychological acute stressors stimulates the release of endogenous Hsp72 into the blood and this elevated Hsp72 functions to facilitate innate immunity in the presence of bacterial challenge (Asea 2005; Feibbraio and Koukoulas 2000; Flesher and Johnson 2005; González and Manso 2004; Johnson and Flesher 2005; Lancaster and Feibbraio 2005; Whitham and Fortes 2008).

The aim of this study was to determine the mechanism by which adaptogens increase endurance in stress. Here we demonstrate that ADAPT-232, which consists of a fixed combination of extracts of three most efficient adaptogens (Rhodiola rosea, Eleuthero-coccus senticosus and Schisandra chinensis) greatly potentiates the ability of mice to overcome exhaustive physical exercise (forced swimming with additional load) by the significant increase in circulating serum Hsp72 levels.

Materials and methods

Test article

ADAPT-232 forte is a proprietary name of a fixed combination of three genuine (native) extracts of Eleuthero-coccus senticosus (Rupr. et Maxim) Harms root, Schisandra chinensis (Turcz) Baill., root, Rhodiola rosea L., root and vitamin B₅, characterised for the content of eleutherosides E and B (0.17%), schisandrin and gamma-schisandrin (0.85%), salidroside (0.33%), tirosol (0.07%), rosavin (0.37%), triandrin (0.01%) and calcium panthotenate (42.8%). The preparation containing a combination of proprietary Rhodiola soft extract SHR-5 (drug extract ratio 2:1; extraction solvent 70% ethanol), Eleuthero-coccus soft extract SHE-3 (drug extract ratio 11.5:1; extraction solvent 70% ethanol) and Schisandra soft extract SHS-2 (drug extract ratio 2.4:1; extraction solvent 95% ethanol), 24% of water and 2% of marker compounds was used in this study. The amounts of the active ingredients rhodioloside, rosavin, tirosol, triandrin, eleutheroside B, eleutheroside E, schizandrin and γ-schizandrin were determined by analytical RP-HPLC using an acetonitrile–water gradient system as mobile phase. Peaks were detected by UV-PAD and analytes were quantified at 221 nm (rhodioloside and tirosol), 252 nm (rosavin), 262 nm (triandrin), 220 nm (eleutheroside B), 210 nm (eleutheroside E, schizandrin and γ-schizandrin). Analytical methods were validated for selectivity, peak purity, precision (RSD < 5%) and accuracy in the range 50–150% of the target amounts of analytes in the tablets in accordance with ICH guidelines (ICH 1996) using Effi Validation 3 software (version 1.03) for testing and calibration laboratories subject to EN ISO/IEC 17 025:2001 (EN ISO 2001).

Animals

Eight- to 10-week-old female BALB/c mice (18.0 ± 0.1 g) were purchased from Taconic Farms (Germantown, NY) and housed in laminar flow isolation units in the Scott and White Memorial Hospital and Clinic vivarium under alternate dark and light cycles. The animals were housed five per cage in a pathogen-free environment with air filter tops in filtered laminar flow hoods. ADAPT-232 forte was dissolved in the drinking water at various concentrations (0, 10, 30 and 60 mg/ml), and mice were allowed to drink it freely. Animals were maintained on food and allowed to drink and eat ad libitum for seven consecutive days. Assuming that daily consumption of water is about 150 ml/kg (Harkness and Wagner 1989), daily doses of active markers were 0, 30, 90 and 180 mg/kg or 0.3, 1.0 and 2.0 g/kg of native ADAPT-232. All animals were treated...
humanely and in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals of the Institute of Animal Resources, National Research Council and Scott and White Memorial Hospital and Clinic.

**Time taken to exhaustion (TTE) measurements**

Animals were placed in a transparent bucket filled with water at ambient temperature and allowed to swim for 30 min. After this animals were dried and a weight equivalent to 6% body weight was attached to the tail and animals placed back in the water bucket. The time taken to exhaustion (TTE) was recorded as the time taken at which the animal stopped swimming. Animals were then taken out of the water, dried and placed into the respective cage.

**Hsp72 enzyme linked immunosorbent assay (ELISA)**

At various times blood was drawn by making a slight cut on the lateral tail vein and circulating serum Hsp72 measured using the classical Hsp70 ELISA as previously mentioned (Bausero et al. 2005). Briefly, drawn blood was allowed to coagulate at room temperature and then centrifuged at 300g for 5 min. Serum-rich supernatant was withdrawn and placed into a fresh eppendorf tube. The total protein content of the serum samples was determined by Bradford analysis using bovine serum albumin as a standard (Bausero et al. 2005). The serum samples were aliquoted and treated with 1% Lubrol WX for 10 min at 4°C with gentle rocking and Hsp72 content was measured by standard sandwich ELISA as previously described (Bausero et al. 2005). Briefly, 96-well microtitre plates (Nunc Immunoplate Maxisorp; Life Technologies) were coated with murine monoclonal anti-human Hsp70 (clone C92F3A-5; StressGen) in carbonate buffer, pH 9.5 (2 μg/ml) overnight at 4°C. Plates were then washed with PBS containing 1% Tween 20 (PBS-T) and blocked by incubation with 1% bovine serum albumin in PBS-T. Supernatant was added and bound Hsp72 was detected by the addition of rabbit polyclonal anti-Hsp70 antibody (SPA-812; StressGen). Bound polyclonal antibody was detected with alkaline phosphatase-conjugated murine monoclonal antibody to rabbit immunoglobulins (Sigma Chemical Co), followed by p-nitrophenyl phosphate substrate (Sigma Chemical Co). The resultant absorbance was measured at 405 nm with a BioRad Benmark Plus plate reader. Standard dose–response curves were generated in parallel with Hsp70 (0–20,000 ng/ml; StressGen), and the concentrations of Hsp70 were determined by reference to these standard curves with ASSAYZAP data analysis software (BIOSOFT). The interassay variability of the Hsp70 immunoassays was <10%.

**Results**

Two series of experiments were curried out with mice taking ADAPT-232 forte in concentrations of 10, 30 and 60 mg/ml for seven consecutive days followed by forced swimming test to exhaustion. Table 1 shows that ADAPT-232 forte strongly augments endurance of mice, increasing the time taken to exhaustion (TTE) in a dose-dependent manner from 3.0 ± 0.5 to 21.1 ± 1.7 min, approximately a seven-fold increase.

Serum Hsp72 was measured both in normal and stressful conditions before and after swimming test (Fig. 1). Fig. 1 shows that repeated administration of adaptogen dose dependently increases basal level of Hsp72 in serum of mice from 0.8–1.5 to 5.5–6.3 pg/ml (lines b–d). This effect is significantly stronger than the effect of stress, including both physical (swimming) and emotional impacts: 3.2 ± 1.2 pg/ml, line e in Fig. 1. Cumulative effect of stress and adaptogen was clearly observed in groups of animals treated with adaptogen after swimming to exhaustion, where serum Hsp72 was measured both in normal and stressful conditions before and after swimming test (Fig. 1). The serum Hsp72 concentration (pg/ml) on respective days post-treatment, groups: (a) control, (b) ADAPT-232 forte, 10 mg/ml, (c) ADAPT-232 forte, 30 mg/ml, (d) ADAPT-232 forte, 60 mg/ml, (e) control + stress, (f) ADAPT-232 forte (10 mg/ml) + stress, (g) ADAPT-232 forte (30 mg/ml) + stress and (h) ADAPT-232 forte (60 mg/ml) + stress.

**Table 1. Effect of ADAPT-232 forte on endurance of mice in swimming test.**

<table>
<thead>
<tr>
<th>Treatment groups (n = 5), concentration</th>
<th>Time taken to exhaustion (TTE) in minutes ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. #1</td>
</tr>
<tr>
<td>Control, water</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>ADAPT-232 forte, 10 mg/ml</td>
<td>8.2 ± 0.9*</td>
</tr>
<tr>
<td>ADAPT-232 forte, 30 mg/ml</td>
<td>18.2 ± 1.8**</td>
</tr>
<tr>
<td>ADAPT-232 forte, 60 mg/ml</td>
<td>19.4 ± 1.6**</td>
</tr>
</tbody>
</table>

*p<0.05 vs control group. **p<0.01 vs control group.

**Fig. 1.** Serum Hsp72 concentration (pg/ml) on respective days post-treatment, groups: (a) control, (b) ADAPT-232 forte, 10 mg/ml, (c) ADAPT-232 forte (30 mg/ml), (d) ADAPT-232 forte (60 mg/ml), (e) control + stress, (f) ADAPT-232 forte (10 mg/ml) + stress, (g) ADAPT-232 forte (30 mg/ml) + stress and (h) ADAPT-232 forte (60 mg/ml) + stress.
increased up to 15.1 ± 1 pg/ml and remained at almost the same level during the 7 days.

Discussion

The data presented in this study demonstrate that the adaptogen, ADAPT-232 forte, strongly augments endurance of mice, increasing the time taken to exhaustion (TTE) in a dose-dependent manner approximately seven fold, by a mechanism, in part, dependent on the release of Hsp72 into systemic circulation. This is a significant finding because of current interest in the role of circulating serum Hsp72 as a danger signal and its biological significance to human physiology (Asea and De Maio 2007). Indeed, there is now a great interest in finding agents that can augment the increase in circulating Hsp72 for therapeutic gain in various human diseases. A recent example is the compound STA-4783 (Synta Pharmaceuticals, Boston, MA), which has been shown to function, in part, by inducing the release of Hsp72 from tumors. In a double-blind, randomized, controlled Phase 2b melanoma study conducted in 21 centers in the US, patients treated with STA-4783, in combination with Paclitaxel, demonstrated significantly increased serum Hsp72 levels, decreased the extent of metastasis and increased the doubling of progression-free survival (Third International Melanoma Research Congress, Noordwijk, The Netherlands, September 2006). Studies are now ongoing to determine the efficacy of ADAPT-232 forte in various tumor animal models (Kaur et al., in preparation).

In this study, we chose to mix the adaptogen (ADAPT-232 forte) into the drinking water and allow animals to drink ad libitum instead of administering the adaptogen using the classical gavage feeding technique, since this causes a slight albeit significant stress to the animals (Roberts et al. 1995). Close monitoring of the body weight of each of the five mice housed per cage did not reveal any significant change in body weight ($p < 0.01$), which might have suggested that one mouse drank more of the mixture than the others. Therefore, we are confident that this technique neither introduces a confounding factor to our studies, nor will it negate the significance of our findings.

In our experiments in mice, we demonstrated that adaptogens induce increase of serum Hsp72, regarded as a defense response to stress, and increase tolerance to stress (in our model combination of physical and emotional stresses). Our data suggest that increased tolerance to stress induced by adaptogen is associated with its stimulation of expression of circulating serum

![Fig. 2. Schematic representation for the hypothetical molecular mechanism by which ADAPT-232 exerts its stress resistance and longevity. In the absence or presence of harmful stressful stimuli, ADAPT-232 forte induces the upregulation of intracellular Hsp70 expression, which in turn inhibits detrimental signal transduction cascades activated by stress, e.g., JNK-induced apoptosis activated by JKK. The decrease in JNK-1 results in the phosphorylation of DAF-16. This allows some of the more favorable functions of JNK to be initiated, including Jun-dependent transcription and the phosphorylation cytoplasmic DAF-16/forkhead transcription factor, which then translocates to the nucleus and synthesizes additional proteins to confer stress resistance and increased longevity.](image-url)
Hsp72 and particularly with Hsp72 production and release, which is known as a mediator of stress response involved in reparation of proteins during physical load. We hypothesize that this could be one of the mechanisms of action of plant adaptogens. Studies from the Sherman laboratory have demonstrated that elevated levels of intracellular Hsp70 inhibit a signal transduction pathway, leading to programmed cell death by preventing the activation of stress-induced activation of Jun N-terminal kinase (JNK, a very early stage of apoptotic process; Gabai et al. 1997, 1998). Recently, we demonstrated that adaptogens, including Schisandra chinensis, inhibit stress-induced JNK expression in rabbits (Panossian et al. 2007). These results are in line with other publications related to the effect of adaptogens on Hsp70 and DAF-16/forkhead transcription factor (Ip et al. 2001; Chiu and Ko 2004; Wiegant et al. 2008a, b). DAF-16, a FOXO-family transcription factor, influences the rate of ageing of Caenorhabditis elegans in response to insulin/insulin-like growth factor 1 (IGF-1) signalling. Our hypothetical model proposes that ADAPT-232 forte exerts its beneficial effects via the stimulation of the stress response (Fig. 2). In the absence or presence of harmful stressful stimuli, ADAPT-232 induces the upregulation of intracellular Hsp70 expression, which is transported to the extracellular milieu and exerts immunostimulatory effects on the hosts’ immune system. Inside the cell, increased Hsp72 expression inhibits detrimental signal transduction cascades activated by stress, e.g., JNK-induced apoptosis activated by JKK. This allows some of the more favorable functions of JNK to be initiated, including Jun-dependent transcription and the phosphorylation cytoplasmic DAF-16/forkhead transcription factor, which then translocates to the nucleus and synthesizes additional proteins to confer stress resistance and longevity (Fig. 2). Our hypothesis is that adaptogens adapt (make less sensitive) the organism to stress, acting somewhat like low molecular weight “vaccines” or stress mimetics, inducing mild activation of stress system in order to cope more severe stress. It should be mentioned that principally, Hsp 72 inducers could have a therapeutic application in the treatment of neurodegenerative diseases (Westerheide and Morimoto 2005).

References


