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Manipulating heat shock protein expression in laboratory animals

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Abstract

Upregulation of heat shock proteins (Hsps) has been observed to impart resistance to a wide variety of physical and chemical insults. Elucidation of the role of Hsps in cellular defense processes depends, in part, on the ability to manipulate Hsp expression in laboratory animals. Simple methods of inducing whole body hyperthermia, such as warm water immersion or heating pad application, are effective in producing generalized expression of Hsps. Hsps can be upregulated locally with focused direct or indirect heating, such as with ultrasound or with laser or microwave radiation. Increased Hsp expression in response to toxic doses of xenobiotics has been commonly observed. Some pharmacologic agents are capable of altering Hsps more specifically by affecting processes involved in Hsp regulation. Gene manipulation offers the ability to selectively increase or decrease individual Hsps. Knockout mouse strains and Hsp-overexpressing transgenics have been used successfully to examine the role of specific Hsps in protection against hyperthermia, chemical insults, and ischemia–reperfusion injury. Gene therapy approaches also offer the possibility of selective alteration of Hsp expression. Some methods of increasing Hsp expression have application in specialized areas of research, such cold response, myocardial protection from exercise, and responses to stressful or traumatic stimuli. Each method of manipulating Hsp expression in laboratory animals has advantages and disadvantages, and selection of the best method depends upon the experimental objectives (e.g., the alteration in Hsp expression needed, its timing, and its location) and resources available.

Keywords: Heat shock proteins; Hsp; Stress proteins; Transgenic animals; 3Knockout animals

1. Introduction

Heat shock proteins (Hsps) are a highly diverse, evolutionarily conserved family of proteins containing both constitutively expressed and stress-inducible members. Hsps are upregulated in response to thermal stress and a wide variety of other physical and chemical insults [1]. Treatments that induce Hsps have been associated with the development of a resistant state where the organism is protected from subsequent toxicity [2], suggesting that increased Hsp expression constitutes an important cellular defense mechanism.

While much of the pioneering work describing the regulation and function of Hsps has been conducted using in vitro models, such as cells in culture, studies indicating a cytoprotective function for Hsps have also been performed in vivo using laboratory animal models. For example, upregulation of Hsps by thermal treatment was shown to provide protection against lethal heat stress in mice [3,4]. Increased Hsps from hyperthermia in rats and mice have also been found to diminish liver injury from hepatotoxicants such as acetaminophen, bromobenzene, and carbon tetrachloride [5–7] and from ischemia–reperfusion [8]. Protection from ischemia–reperfusion injury in the heart has been shown to result

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from upregulation of Hsps through exercise training or pharmacological treatment [9,10].

Given that Hsps are induced in response to a variety of stressors, it is not surprising that an array of techniques has been used to manipulate Hsp expression in vivo for experimental purposes. Beyond the classical stimulus of heat application, several different methods have been utilized to achieve general or selective upregulation of Hsps in laboratory animals. Other methods are available to either preclude or inhibit Hsp expression. The purpose of this paper is to provide an overview of methods available to alter Hsp expression in laboratory animals, discuss changes in expression that have been observed, and where possible, discuss the strengths and limitations of the methods.

2. Description of methods

Most methods to manipulate Hsp expression in laboratory animals can be grouped into physical, pharmacological, and genetic alteration categories. Physical methods include direct or indirect application of heat, both generally and to localized areas. Hsp expression can also be altered through the use of pharmacologic agents. Drugs and chemicals are capable of producing an Hsp response by virtue of their toxicity, and in some cases more selectively by affecting critical processes in the regulation of Hsps. Genetic alteration approaches involve insertion or deletion of genes important for Hsp expression through a variety of means. Other methods, such as exercise, cold treatment, and psychological stress have been found to increase Hsp expression in laboratory animals. Examples of these methods are described in the following sections.

2.1. Physical methods

2.1.1. Water immersion

Immersion of anesthetized mice in a warm water bath has been used successfully by our laboratory to increase the expression of Hsps in the liver. Mice are anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in nylon mesh vests with openings for the legs. The vests are equipped with velcro closures and fitted with a 1 oz lead weight on the lower back and an attachment hook on the anterior ventral side. The weighted vest allows the anesthetized animal to remain upright when suspended in the water bath to avoid drowning. Mice are immersed mid-sternum in a rapidly recirculating water bath (41.5 °C water for 30 min) to obtain a core body temperature between 41.0 and 41.5 °C for 20 min, as monitored by rectal temperature probe (Fig. 1). Control animals are anesthetized and handled in a similar manner but receive no water bath treatment. Livers harvested 48 h after heat treatment show Hsp25 and inducible Hsp70 expression

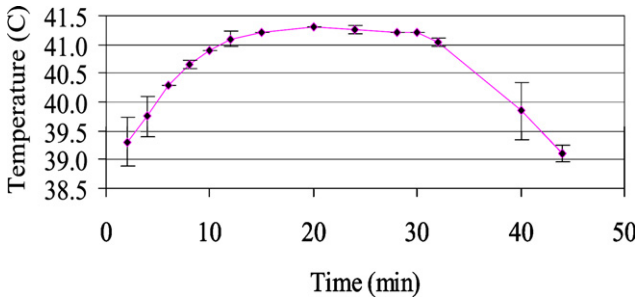

Fig. 1. Core body temperatures in mice during thermal stress treatment using water immersion. Mice were immersed in a recirculating water bath (41.5 °C) for 30 min, and core body temperature was measured using a rectal probe.

strongly increased above control levels (Fig. 2). Expression of Hsp110 and Hsp32 is moderately increased, while no change in expression of Hsp60 and Hsp90 (not shown) is observed. Upregulation of Hsp expression is seen as early as 6 h after heating and persists for as long as 96 h. Following treatment, mice recover quite slowly from anesthesia, and normal food and hydration levels remain suppressed for an additional 24 h. Consequently, mice should be allowed to recover for a full 48–72 h prior to further experimentation.

2.1.2. Heating pad

Upregulation of Hsps from whole-body heating has also been accomplished using heating pads. In one study, mice were anesthetized with 2.5% Avertin in saline (20 μL/g body weight by injection) [4]. Animals were wrapped in a heating pad, and rectal temperature was monitored to maintain 41 ± 0.1 °C for 30 min. After a 48-h recovery period, mice were subjected to a lethal heat

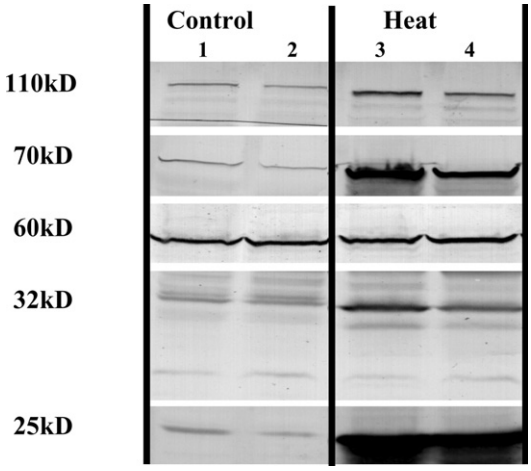

Fig. 2. Hsp induction in liver following water immersion thermal treatment. Lanes 1 and 2 were from control (unheated) animals. Lanes 3 and 4 were from animals thermally treated by immersion in a heat water bath (41.5 °C) for 30 min. Following thermal stress, mice were allowed to recover for 48 h prior to harvesting livers for SDS–PAGE and Western blot analysis for Hsp induction.