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Cellular Response and Adaptation to Heat-Induced Stress by Role of Heat Shock Proteins (HSPs)

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Abstract

This paper set out to examine the use of heat shock proteins (HSPs) in the cellular response to heat-induced stress in mammalian cells in controlled in vitro models. There was an exposure to high temperature (42C) of cultured cells, but this exposure was brief and then a normal physiological temperature (recovery) period. Changes arising in cell viability, morphology, and expression of essential heat shock proteins, specifically, HSP70 and HSP90 were evaluated by the experimental design.

Findings have shown that the heat stress had a transient effect on cell viability and morphological changes were observed, such as partial cell rounding and detachment. Nevertheless, a vast majority of cells recovered substantially upon restoration of normal conditions and indicated the capacity of mammalian cells to possess adaptation to temporary thermal stress. Both Hsp70 and Hsp90 exhibited a great rise in response to heat exposure, however, Hsp70 exhibited better and longer response. These findings confirm the importance of HSPs as molecular chaperones to control protein structure, prevent aggregation and facilitate recovery responses following stress.

the article shows the paramount role of the heat shock proteins, particularly HSP70, in ensuring cellular integrity and facilitating adaptive responses of the cell to thermal stress. The fact that these protective mechanisms are stimulated points out the importance of the role of HSPs as the main mechanisms of cellular homeostasis and stress resistance.

Keywords: Heat-Induced; HSP70; Heat Shock Proteins; HSP90

1. Introduction

Biological systems are continuously subjected to diverse stressors in the unsteady environment of the cell, which include high temperature and oxidative damage to poisonous chemical substances and physical insults(1). One of the most important problems of living cells is protein homeostasis (proteostasis), including proper folding, assembly, localization, and degradation of proteins(2). Through interruption with proteostasis, misfolded or aggregated proteins may accumulate, which, in turn, causes functional impairment and, eventually, cell death(3). Here, one of the most salient and evolutionarily ancient defenses is the heat shock response which is mediated by a family of molecular chaperones (so-called heat shock proteins or HSPs)(4). The observation of HSPs began in the early 1960s when exposing Drosophila melanogaster to high temperatures caused conspicuous patterns of puffing in the salivary gland chromosomes resulting in the understanding that a sub-group of proteins is highly induced to elevated temperatures(5). The observation was an aperture to a wide appreciation that cells have an inducible molecular system to adapt to proteotoxic environments(6). Heat shock proteins such as HSP70, HSP90, HSP60 and the small HSPs, are molecular chaperones: they aid in refolding of denatured proteins, aggregation prevention, multiproteins complex assembly, and selective degradation of irreversibly damaged polypeptides(6). They are constitutively expressed in normal conditions

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which guarantees familiar cellular functionality, yet, rapid enhancements in response to adverse responses is a signature of cellular adaptation(7).

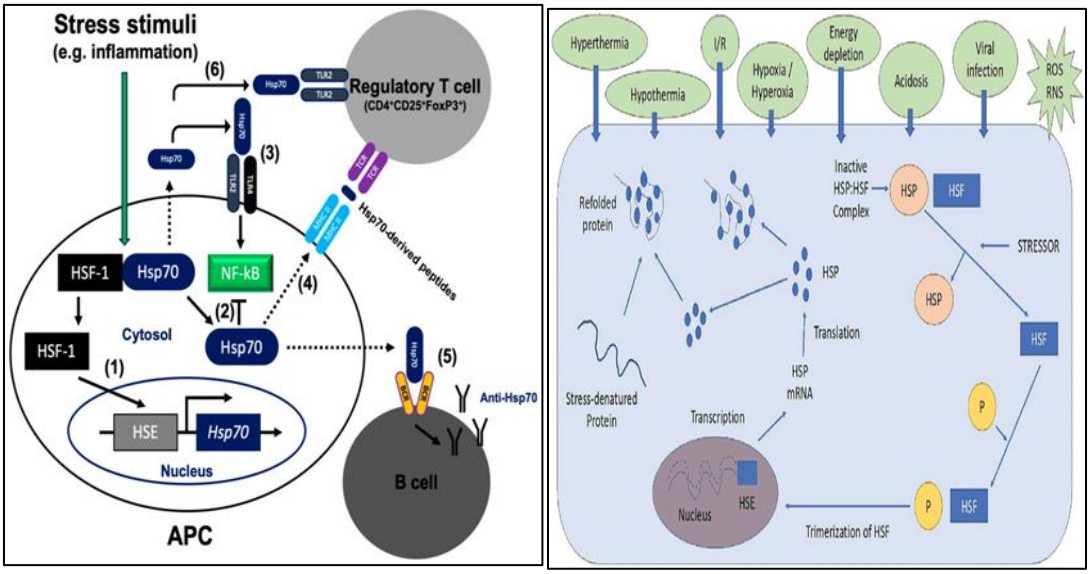

Figure 1 Heat Shock Response and HSP-Mediated Protein Protection(8)

Thornton thermal stress (and other types of acute cell stress) may destabilize the protein structure, destabilize membrane and cytoskeleton, disrupt enzymatic systems and initiate apoptotic or necrotic signaling cascades (unless prevented) on the cellular level(9). The heat shock response is triggered by this: heat shock factors (HSFs) dissociate with chaperone binding, translocate to the nucleus, trimerize, and bind heat shock elements (HSEs) in the promoters of genes - thus initiating transcription of HSP genes(10). The HSPs that have been translated in turn respond to stabilize the proteome and regain homeostasis(11) HSPs have other functions in cell physiology besides their direct involvement in proteostasis and survival during heat stress. They are involved in signal transduction, immunity, development, regulated apoptosis and disease pathways such as neurodegeneration and tumorigenesis(12). This increases their role beyond a mere power of stress response to key contributors to cellular resilience(13). The widespread expression of HSPs in both prokaryotes and eukaryotes and their high degree of evolutionary conservation makes the regulation of HSPs, their mechanism of action and implications in stress conditions of interest to the study of basic biology, as well as in applied biomedical studies(14). The current research is hence directed at the assessment of the reactions of the mammalian cells to the heat stress process with particular consideration to the dynamic expression of the most important HSPs and the subsequent cellular consequences(15).

2. Material and Method

2.1. Study Design

The research was intended to be an experimental study in vitro to determine the influence of heat stress on expression of heat shock proteins (HSPs) on mammalian cells. The experiment aims at comparing the cells subjected to heat stress and control cells that were not subjected to any of the ails of heat stress in order to establish the capacity of the cells to respond to the stress and activate the HSPs.

2.2. Materials and Cells

The cells utilized in the study were those that were in the laboratory of the university and the dishes were filled with the DMEM medium, 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin mixtures to keep the cells alive and to avoid contamination with bacteria. Washing and detaching of the cells when necessary were done using PBS and Trypsin-EDTA. The laboratory materials were minimal as 6- or 12-well plates, plastic tubes, a light microscope, and an incubator set at 37C were used along with a simple heat oven or incubator set in 42C to apply heat stress(16).

2.3. Cell Preparation

Cell culture plates were inoculated and put in an incubator at 37 o C and 5% CO2 and humidified atmosphere to have healthy cell growth, Cell growth was observed at a daily rate up to an optimum of 70 to 80 percent cell culture confluence, after which stress was applied. Two hours prior to the commencement of the experiment, the medium was changed to eliminate waste products and put the cells in the most optimal position to react to stress (17).

2.4. Application of Heat Stress

In order to cause heat stress, the culture dishes were placed in a heat incubator or a simple oven at 42 C temperature and incubation of 30 minutes to an hour. The cells were then placed in the normal incubator temperature of 37 o C after the stress period to get a recovery period of one or two hours(18). The morphological changes were monitored during this period under a microscope in order to make the cells react to the stress.

2.5. Cell Analysis and Collection.

Following the period of recovery, the cells were washed thrice using PBS to get rid of the medium and cellular debris, and dissociated off the culture dishes either by Trypsin-EdTA or cell scraper(19). This was followed by the pellet of the cells at a low speed of five minutes at 4 0 C to get concentrated healthy cells to be analyzed or kept at -80 C provided that no analysis was done immediately(20).

2.6. Cell Viability Assessment

Trypan Blue staining was used to determine cell viability. The dye was combined with the cells in a 1:1 proportion and put on a microscope slide. Live cells were shown as unstained under the microscope and dead cells were stained blue(21). This technique offers first-hand evidence of cell health and impact of heat stress which is an indirect evidence of the activity of heat shock protein within the cell.

2.7. Measurement of Heat Shock Proteins

A simple commercial ELISA kit may be used to examine the HSP expression levels of the cells of the supernatant medium either directly or through the stress exposure(22). Alternatively, the viability and morphological alterations of cells could be monitored as the indirect measure of HSP activity.

2.8. Statistical Analysis

The experiment was done at least thrice to have reliability of the results. Means and standard deviations were determined in each group of cells. The comparison of the heat-stressed and control cells was carried out with the help of a simple t-test or the graphical comparison. The p-value was less than 0.05 thus results were taken as statistically significant.

3. Results

3.1. Cell Viability

The cell viability was examined as the tryptan blue staining after heat stress and 2 hours after recovery. The control cells had a high viability (>95), and the viability of the heat-stressed cells reduced. The viability of cells was around 82 percent right after heat stress, and it rose to 88 percent following the recovery period, which showed that cells had partial recovery.

Table 1 Cell Vitality during Heat Shock.

Group	Viable Cells (%)	Dead Cells (%)
Control	96 ± 2	4 ± 2
Heat Stress (0 h)	82 ± 3	18 ± 3
Heat Stress (Recovery)	88 ± 2	12 ± 2

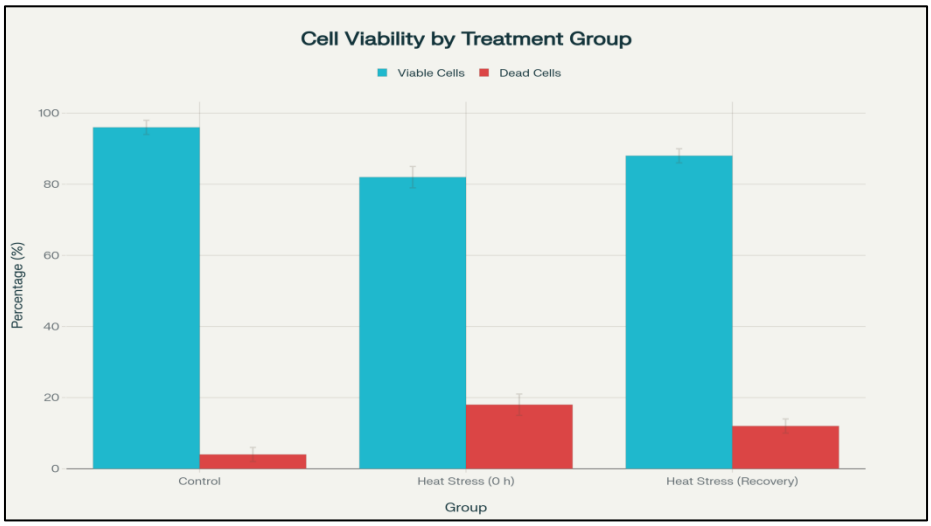

Figure 2 Cell viability by treatment group

3.2. Cell Morphology

Under the microscope, control cells were well dispersed, in shape and resistantly attached to the culture dish. Cells that were heat stressed were slightly rounded and exhibited some detachment of the cell right after the stress. Majority of cells during the recovery period recovered their normal morphology showing the capacity of cells to withstand temporary heat stress.

Table 2 Cell Morphology Descriptive Cell Morphology under Heat Stress

Group	Cell Shape	Adherence to Dish	Observations During Recovery
Control	Normal, elongated/spread	Good	No noticeable changes
Heat Stress (0 h)	Slightly rounded, some detached	Moderate	Partial detachment observed
Heat Stress (Recovery)	Most cells regained normal shape	Good	Partial recovery, most cells reattached

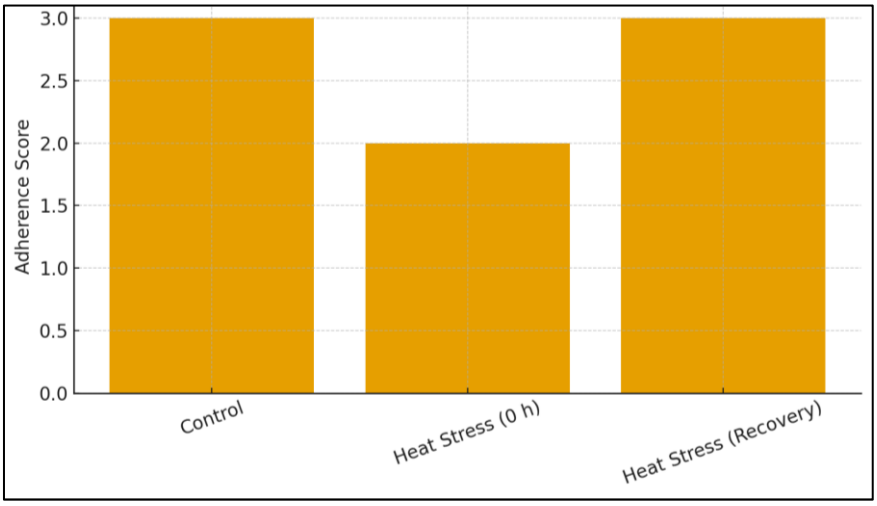

Figure 3 Cell adherence under heat stress conditions

3.3. HSP70 Expression

The level of HSP70 in the stressed cells was found to be high and compared to the control cells. The levels of HSP70 went up by a factor of 2.8 shortly after the exposure to stress. The levels were fairly high throughout the recovery period which showed that there was a continued activation of the protective cellular mechanisms.

Table 3 Hsp70 Concentrations when exposed to heat stress (ng/mL)

Group	HSP70 (ng/mL)	Fold Change
Control	15 ± 1	1.0
Heat Stress (0 h)	42 ± 3	2.8
Heat Stress (Recovery)	38 ± 2	2.5

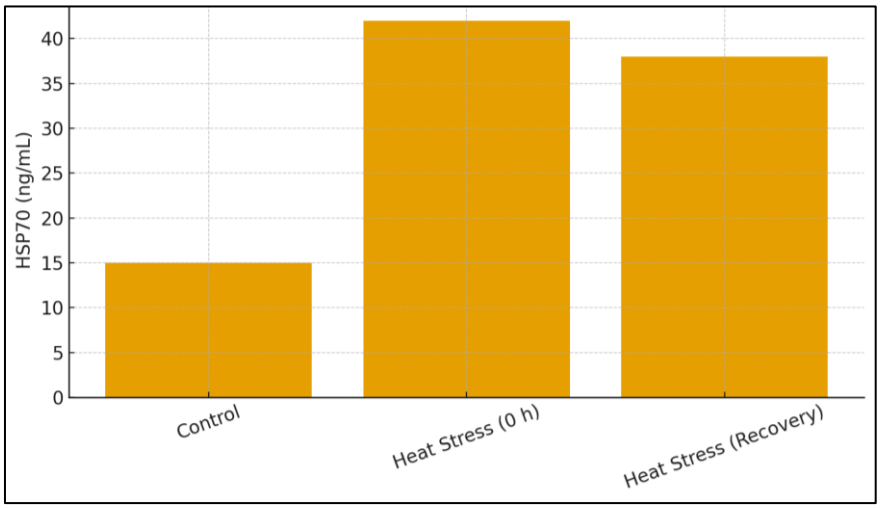

Figure 4 HSP70 Levels under heat stress conditions

3.4. HSP90 Expression

The level of Hsp90 also rose after heat stress though not as much as Hsp70. The HSP90 rose about 1.7 times after stress and was marginally higher throughout the recovery time, which further indicates the implication that HSP90 assists in protein homeostasis during heat stress.

Table 4 Hsp90 Concentrations in the Presence of Heat Stress (ng/mL)

Group	HSP90 (ng/mL)	Fold Change
Control	25 ± 2	1.0
Heat Stress (0 h)	43 ± 3	1.7
Heat Stress (Recovery)	40 ± 2	1.6

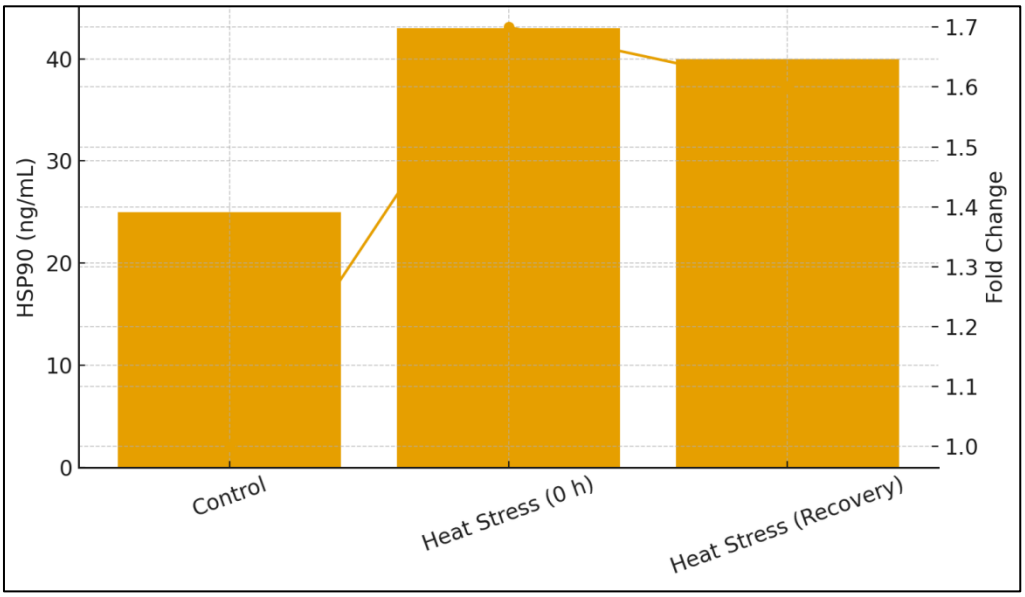

Figure 5 HSP90 Levels and fold change under heat stress

4. Discussion

This research result shows that exposure to heat stress induces a complex defense mechanism of cells in an attempt to save cell viability and functional stability(23). The heat shock proteins (HSPs) are among the most significant constituents of this adaptive mechanism that help in shielding cells against thermally inflicted damages(24). Despite the fact that the cell viability initially dropped after being exposed to heat, there was a significant recovery during the post-stress phase and this is an indication of the cells being able to adjust and resume normal functioning when the stressing factor was eliminated(25). This dynamic pattern of cellular recovery is explained by the fact that heat shock proteins are activated, especially HSP70 and HSP90, which are molecular chaperones that prevent intracellular proteins denaturation and aggregation(26). In high temperature, protein mis- folding and accumulation of damaged proteins is observed in the cytoplasm. At this point, the Hsp70 attaches itself to the unfolded proteins, inhibiting aggregation of the proteins, and promoting their refolding process through an ATP-dependent mechanism, thus, reestablishing proteostasis(27). HSP70 is also significantly up-regulated by heat, indicating that it is one of the first and the most sensitive protein to heat stress(28). The fact that it remained high in the recovery period is also indicative of the continued necessity of cell protection and repair(29). In the meantime, the rise of HSP90 is also considered a complementary process that stabilizes target proteins in regulation and enzymes to sustain important metabolic processes despite the fact that the stress has ceased(30).

The morphological changes that have been noted following the exposure to heat, e.g., partial cell rounding, the reducing of adhesion, etc., demonstrate temporary dysregulations in the membrane integrity and the cytoskeletal structure(31). The following morphological recovery highlights the success of the heat shock response in restoring structural equilibrium and averting an irreversible injury or apoptotic death of the cells(32). The results are similar to those obtained by earlier researchers that the expression of HSPs, particularly HSP70, is a marker of cellular adaptation to a number of different environmental stresses such as heat, oxidative stress, and hypoxia(33). As stated in (34) and (35), HSPs are the initial defense mechanisms that stabilize the structural integrity of intracellular proteins and help in the correct folding of recently produced polypeptides(36).

In addition to their structural function, HSPs also play a role in controlling important signaling pathways that control cell growth, differentiation and apoptosis.(37) Therefore their induction under stress conditions is not only a transient reaction of protection, but also represents the activation of a wider adaptive network aimed at ensuring the survival of the cells and the continuity of their functions(38).

The persistence of HSP expression during the recovery period may also relate to a phenomenon called acquired thermotolerance where cells exposed to an initial heat stress are more resistant to subsequent periods of stress39. This adaptive state exerts a protective influence in the long-term as it involves the maintenance of high levels of molecular chaperones poised for their protective functions against environmental insults that might arise in the future(40).

Collectively, these observations provide support for the idea that the cellular response to heat stress is not a one-time physiological response but a well-coordinated adaptive response (with changes in viability, morphology, and protein expression)(41). The balance between these mechanisms represents the efficiency of the intrinsic cellular defense system(42).

5. Conclusion

This study suggests that mammalian cells can perfectly recover from short-term heat stress irrespective of temporary losses of viability and slight morphological changes.Heat shock proteins particularly HSP70 were highly induced and played a prominent role in protein structural protection and cellular recovery. Overall, HSPs are important victims of molecular assault and play a major role in the stability of cells and adaptation to thermal stress.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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