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# Cadmium Toxicity and Cell Stress Response

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**Abstract:** Cadmium is a potent toxicant that is linked to multiple human disorders. Recently, it has been shown that cadmium induces the expression of heat shock protein 70 (HSP70) and polyubiquitin genes in the fresh-water snail, *Biomphalaria arabica*. In this report, the hazarodus effects of cadmium, the mechanism of cadmium-mediated cell stress response, and the use of cell stress response as a bio-indicatior for cadmium toxicity are discused.

**Key word:** Cadmium, heat shock proteins, ubiquitin

## Cadmium toxicity

Cadmium is extremely hazardous to life and has been involved in historic poisoning episodes of human and animal populations. It is a serious lethal occupational and environmental toxin, known for its high toxicity, which may affect living systems in various ways (Ostrowski *et al*., 1999). Recently, it has been ranked number 7 among top 20 Hazardous Substances in Priority List of the Agency for Toxic Substances and Disease Registry/Environmental Protection Agency in 1997 (Ostrowski *et al*., 1999).

Cadmium is primarily used in plating iron and steel to prevent corrosion, and manufacturing of nickel-cadmium batteries. The metal is also used in plastics, ceramics, paints and various solder and brazing alloys. Moreover, cadmium is used in solar cells, television tubes, lasers, and cadmium telluride devices prepared by semiconductor manufacturers.

Cadmium exposure occurs through air, water, food and dust. It accumulates slowly in the body for 50 to 60 years (Bernard, 1986). Cadmium is deposited in liver, muscles, lungs and kidney tissues. About 50 percent of the body burden of cadmium is present in the kidneys and the rest is distributed to liver and other muscles (Kazantzis, 1987). Acute cadmium toxicity has been known to result via ingestion of cadmium-containing food in which the metal is bound to the protein metallothionein (Cd-Mt) (Pascoe *et al*., 1996). Severe gastrointestinal problems, including abdominal pain, nausea and vomiting, along with systemic shock reaction, have been reported. Chronic exposure to low levels of cadmium by inhalation route may lead to respiratory disease such as emphysema, renal and neurological disorders such as Alzheimer's disease (Basun *et al*., 1991; Nemery, 1990).

The International Agency for Research on Cancer (IARC) has classified cadmium as agroup 1 human carcinogen of prostate and lung. A significant increase in prostate cancer has been reported in factory workers of cadmium-nickel batteries in England and United States (Kazantzis, 1987).

At cellular level, cadmium exposure leads to protein denaturation, DNA strand breaks, and formation of reactive oxygen species and lipid peroxidation. Cellular response to the metal exposure includes the transcription of diverse genes that encode repair and defense proteins. This includes heme oxygenase (Sunderman, 1987), metallothionein (Hamer, 1986), α-glutamylcysteine synthetase (Hatcher *et al*., 1995), heat shock proteins (Wiegant *et al*., 1994) and ubiquitin (Muller-Taubenberger *et al*., 1988). These proteins ensure prevention of further protein damage, removal of reactive oxygen species, DNA repair and degrade or/and renature of misfolded proteins.

**Cell Stress Response:** Cell stress response was first discovered in 1962 (Ritossa, 1962). It was observed that a distinct pattern of the Drosophila salivary gland chromosome, called chromosomal puffs are induced in response to transient exposure of the insect to elevated temperatures. Now, it is evident that cell stress response is ubiquitous and highly conserved in all orgamisms from bacteria to man. It is a necessary mechnism for protection of cells from harmful condtions, including heat shock, heavy metals, oxidative stress, alcohol, inflammation or fever (Lindquist, 1986; Morimoto, 1993).

Cell stress response is usually modulated at the molecular level via two distinct mechnisms. The first mechanism ensures the synthesis of cell stress proteins or so-called heat shock proteins (HSPs), which function as molecular chaperones to assist protein folding, translocation and refolding of intermediates (Georgopoulos & Welch, 1993). The second mechanism ensures the synthesis of proteases and hydrolytic components of the ubiquitin-dependent proteasome for degradation of damaged and short-lived proteins (Hershko and Ciechanover, 1992).

According to their molecular size, HSPs are classified into six major families. These are HSP100, HSP90, HSP70, HSP60, HSP40 and small heat shock proteins. Each family of HSPs comprises proteins that are constitutively expressed and/or induced under certain conditions and may be targeted to different compartments. For instance, HSC70 (heat shock cognate 70) and HSP70 proteins are cytosolic and nuclear proteins. Others like GRP78 (glucose-regulated protein 78) is localized to the endoplasmic reticulum and mHSP70 (mitochondrial HSP70)/GRP74 (glucose regulated protein 74) is localized to the mitochondria. These proteins of the HSP70 family exhibit complex patterns of gene regulation over growth and stress, and are targeted to different subcellular compartments. Also members of HSP90 family function in a similar pattern to that of HSP70 family members. For example, HSP90 functions in both the cytosolic and nuclear compartments, whereas GRP94 performs the analogous function in the endoplasmic reticulum.

Regulation of the cell stress response is a remarkable tool to investigate the mechanisms and intradynamics of inducible gene expression in eukaryotes under stressful conditions (Morimoto, 1993). Induction of cell stress genes requires the binding of at least one heat shock factor (HSF) to a specific sequence(s) known as heat shock elements (HSEs), which comprises multiple adjacent inverted arrays of the binding site (5'-nGAAn-3') within the promoters of cell stress genes.

In vertebrates and plants, there are at least four members of

the HSF gene family where, gene induction requires the de novo binding of HSF(s) transiently to the HSE(s) (Wu, 1995; Morimoto, 1998). In *Drosophila melanogaster*, *Saccharomyces cerevisiae* and *Caenorhabditis elegans* a single HSF is expressed.

In human cells, three HSFs (HSF1, HSF2, HSF4) are expressed (Wu, 1995; Morimoto, 1998). Only HSF1 is ubiquitously expressed and play a role in the stress-induction of heat shock genes, whereas, HSF2 is activated during specific stages of development and HSF4 is expressed in specific tissues and may act as an inhibitor of cell stress response. However, HSF3 has only been characterized in avians. Like HSF1, this factor is activated by stress under different conditions.

The heat shock gene family includes a multigene repeates encoding for a highly conserved 76 residue protein known as ubiquitin, which is required for proteasomal degradation of damaged and short-lived proteins (Hershko and Ciechanover, 1992). The primary gene products of ubiquitin genes are all fusion proteins. These fusion proteins must be proteolytically processed to obtain mature ubiquitin. Subsequentlly, ubiquitin monomors are attached to damaged and short-lived proteins for non-lysosomal protein degradation (Hershko and Ciechanover, 1992).

Ubiquitin coding genes are classified into three classes. Class I and II genes encode two proteins, each consists of a single copy of ubiquitin fused to ribosomal proteins (Bishoff and Schwartz, 1990; Baker and Board, 1991; Cabrera, *et al.*, 1992). The class III gene (polyubiqiutin genes) encodes a ubiquitin precursor consisting of head-to-tail repeats of the ubiquitin protein sequence (Wiborg, *et al.*, 1985; Ozkaynak *et al.*, 1987). The number of ubiquitin repeats varies with the organism. The class III of ubiquitin genes contains a consensus heat shock promoter and found to be a heat shock protein (Bond and Schlesinger, 1985; Bond and Schlesinger, 1986; Finley *et al.*, 1987).

Prior to substrate degradation, ubiquitin monomers are conjugated to the substrate in a multiple enzymatic process. he degradation of the substrate requires further the ubiquitination of attached ubiquitin, forming a branch of multiubiquitin chains. This chain is recognized by the 26S proteasome, a huge particle of proteases, leading to degradation. Thus, the substrate specificity for ubiquitination assures its degradation.

**Cadmium-mediated cell stress response:** An important feature of cadmium-mediated cell stress response is the induction of metallothionein, a cellular protein with high efficiency for cadmium binding (Hamer, 1986).

Cadmium also induces the expression of several cell stress genes. The metal has been shown to increase the expression of HSP70 among other cadmium-responsive genes from the nematode, *Caenorhabditis elegans* (Liao and Freedman, 1998). Moreover, cadmium was shown to induce the expression of HSP70 in the marine sponge *Suberites domuncula* (Schroder *et al.*, 1999), murine L929 cells (Liu *et al.*, 1994), HepG2 cells (Salminen *et al.*, 1996), H35 rat hepatoma cells (Ovelgonne *et al.*, 1995a) and 9L rat brain tumor cells (Hung *et al.*, 1998). Also, it was shown to induce HSP70 in human proximal tubule and hepatoma cells (Steiner *et al.*, 1998; Somji *et al.*, 1999). Cadmium also induces the transcription of HSP70 in the fresh water snail, *Biomphalaria arabica*, exposed to lethal and sublethal concentrations of the element (Al-Khedhairy, 2000). The relationship between cadmium and induction of cell stress gene expression is non-linear and affected by cadmium concentration and duration of exposure (Ovelgonne *et al.*, 1995b).

It is indicated that sublethal concentrations of cadmium can inhibit protein synthesis and also increase the synthesis of certain HSPs, among which HSP70 is the most strongly inducible heat shock protein. The pattern of HSP induction changes, when exposure condition becomes more severe. Thus, differential HSP expression takes place, possibly, in all *in vivo* and *in vitro* systems. For instance, cadmium is a potent inducer of HSP70 mRNA, however, it does not induce HSP60 nor its mRNA in H35 rat hepatoma cells (Ovelgonne *et al.*, 1995a, b). Therefore, this differential expression represents a valuable tool for biomonitoring of cadmium toxicity.

Moreover, cadmium induces the expression of polyubiquitin genes. UB14, the polyubiquitin gene of *Saccharomyces cerevisiae*, is strongly induced in response to the metal (Chen *et al.*, 1994). Moreover, three polyubiquitin transcripts of 0.9, 1.2 and 1.4 kb from *Dictyostelium dictyostelium* are overexpressed in response to cadmium treatment (Muller-Taubenberger *et al.*, 1988).

Recently, cadmium was shown to induce the expression of polyubiquitin in *B. arabica* (Al-Khedhairy, 2001). Comparison of both cadmium-induced expression of HSP70

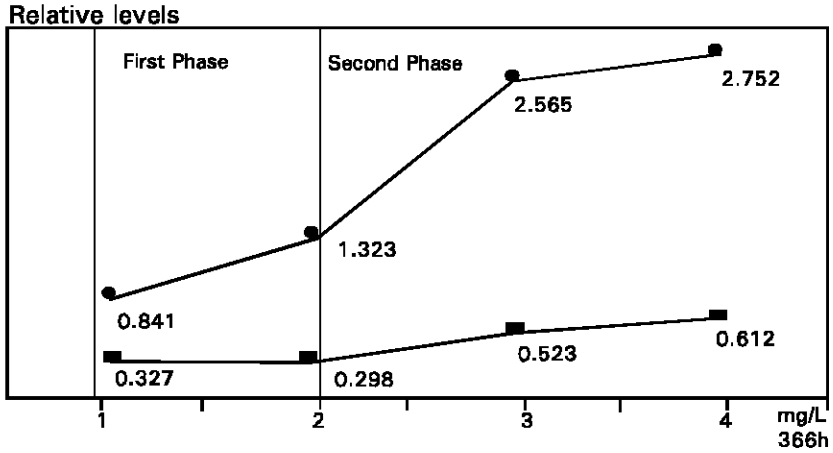


Fig. 1: Cadmium induced expression of HSP70 and polyubiquitin in *B. arabica*. First phase includes only the induction of HSP70 gene expression (●). Second phase includes the induction of the second gene, polyubiquitin (■).

and polyubiquitin indicates. The former is more vulnerable to cadmium toxicity. Thus, the comparison of multiple cell stress gene expression may provide another valuable tool to monitor such toxicity.

At the cellular level, cadmium interacts with thiol groups of proteins and can substitute zinc in certain proteins (Vallee and Ulmer, 1972). Consequently, cellular proteins are abnormally denatured, possibly, through weakening of polar bonds and exposure of hydrophobic residues (Wedler, 1987). Thus, the stimulation of cell stress gene expression may be a consequence of the stress-induced accumulation of cellular proteins (Ananthan *et al.*, 1986; Lee and Hahn, 1988).

Under mild cadmium toxicity, denatured proteins may be refolded by mechanisms of molecular chaperones such as HSP70 (Georgopoulos & Welch, 1993). When cadmium toxicity persists for longer exposure and/or increased concentration, denatured proteins are likely ubiquitinated and subsequently degraded in the 26S proteasome complex (Hershko and Ciechanover, 1992). Thus, induction of polyubiquitin gene expression along with HSP70 may indicate either longer exposure time and/or higher cadmium concentration. This relationship was observed in *B. arabica* exposed to lethal and sublethal concentrations of the element (Al-Khedhairy, 2000; Al-Khedhairy, 2001). Two phases for the induction of cell stress genes can be observed (Fig. 1). First phase includes the induction of HSP70 gene expression whereas; the second phase includes the induction of the second gene (polyubiquitin).

Regulation of gene expression involves the binding of cell stress proteins, i.e. HSP70, to denatured proteins, releasing inactivated HSF from the HSP-HSF complex. Subsequently the factor enters the nucleus and binds to HSE present within the promoter region of most cell stress genes including HSP70 and polyubiquitin, thus inducing these genes (Craig and Gross, 1991; Baler *et al.*,1992; Morimoto, 1993).

An important feature of the fauna of Saudi Arabia is the wide distribution of molluscs over 600 localities within seven regions covering the entire country (Brown & Wright, 1980; Siddiqui, 1981). This molluscan fauna is dominated by *Lymnaea auricularia* (Linnaeus), *Melanoides tuberculata* (Müller) and *B. arabica* (Melvill and Ponsonby), which can be found in all parts of the country in quite large numbers. These species may represent a good choice for use as a biomarker to detect effects of water toxicants before a full–blown toxic responses.

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