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Expression of Stress Proteins Induced by Cadmium "*In Vivo*" and "*In Vitro*" and the Search for the Protective Effect of Magnesium in *In Vitro* Culture

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Abstract

Our results show an over expression of HSP 72/73 in the liver, kidneys and testes. This overpressure serves to protect the body against Cd, and is considered as a biomarker of exposure. The *in-vitro* culture confirms these results where the addition of Cd in the culture medium of A549 cells induces over expression of HSP72. "*In-vitro*", Mg does not modify Cd-induced HSP expression. "*In-vitro*" Mg, does not decrease the cytotoxic effect of Cd, and therefore the protective effect of Mg appears to be interacted with the dynamics of absorption, transport and accumulation of Cd in the body, which lowers or decreases its endogenous exposure rates and consequently its toxic effects.

Keywords: Antioxidant; Biomarqueur; Cadmium; HSP; Oxidative stress

Introduction

Stress is involved in all relationships between the individual and the environment around him. From the moment of birth and at each moment of existence, its consequences can be extremely serious and even put into question the survival of the individual. There exists in the cell, a protection system consisting of proteins called stress or heat shock. Their role is demonstrated in the differentiation of muscle cells, the fight against infections and chemical or toxic stresses as well as the protection against the increase of temperature and hypoxia. For cell survival, particularly under difficult conditions, one of the mechanisms that are most maintained during evolution is that of the expression of proteins known as heat shock proteins, or stress proteins, therefore these proteins are expressed as a result of any situation that compromises cell survival. These situations include temperature rise, exposure to heavy metals or other chemicals such as a zone, hypoxia, infections or lack of glucose. Their role is then to protect the vital set of cellular proteins; indeed they have a capacity to repair other proteins or elimination when they can no longer be repaired. They function as a molecular chaperone accompanying, monitoring and protecting other proteins. Thus, their synthesis can increase in cells in culture by exposure to amino acid analogues, heavy metals [1]. The increase in HSP expression therefore can be considered as a marker of increased stress resulting from environmental pollution [2].

Material and Methods

Animals

Our study is carried out on male white rats of «wistar» strain. These animals are placed in a pet shop where the temperature is close to 23°C alternating between 10 hrs of darkness and 14 hrs of light. The food is given at will; it is a concentration in the form of energy balanced caps from SICO-S fax.

Animal model design

The 1st protocol is performed on 4 groups of rats: Male rats weighing about 120 g are injected intraperitoneally for one day either with NaCl 9% or magnesium sulfate at two different doses 300 mg/kg PC (Mg1 group) and 600 mg/kg PC (group Mg2). The next day, noted jo, rats that are injected with NaCl are divided into two groups, one will be injected throughout the experimental period by the physiological liquid qualifies as control (T group), the other by chloride cadmium at

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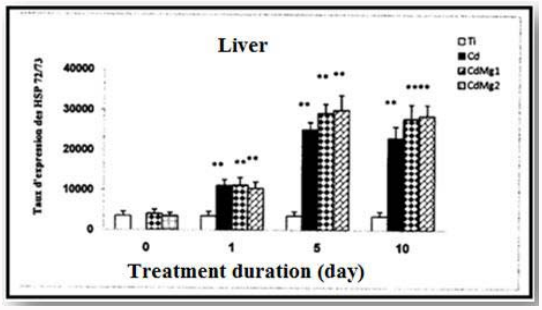

Figure 1: Expression level of HSP72 proteins in the liver of the control (T) and rats injected with cadmium chloride (Cd) alone or in combination with magnesium (CdMg1) and (CdMg2).
The values represent the mean ± ESM (n=8).
**: P ≤ 0.01 compared to the control rats.

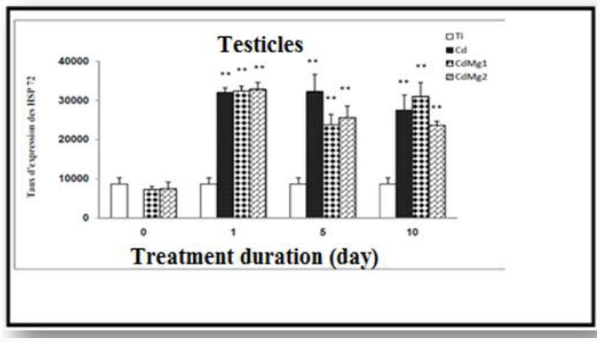

Figure 2: Expression level of HSP72 proteins in testes of rats controls (T) and rats injected with cadmium chloride (Cd) alone or in combination magnesium sulfate (CdMg1) and (CdMg2).
The values represent the mean ± ESM (n=8).
**: P ≤ 0.01 compared to the control rats.

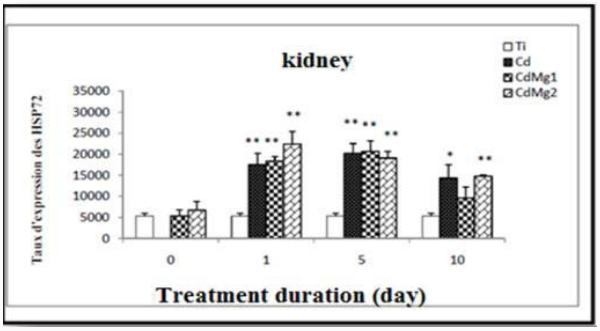

Figure 3: Expression level of HSP72 proteins in the kidneys of rats controls (T) and rats injected with cadmium chloride (Cd) alone or in combination magnesium sulfate (CdMg1) and (CdMg2).
The values represent the mean ± ESM (n=8).
**: P ≤ 0.01 compared to the control rats.
*: P ≤ 0.05 compared with the control rats.

2.5 mg/kg of CP (group Cd). Concerning the Mg1 and Mg2 groups, the same solution of Cd chloride (Cd group Mg1 Cd Mg2) is added there to. The treatment lasts 0.1, 5 and 10 days.

Assays: The HSP 72/73 stress protein assay is performed by electrophoresis and Western blotting. The quantization of the expression of the stress proteins is determined after having assayed the total proteins by the method of Lowry by following the following steps:

1. SDS polyacrylamide gel electrophoresis in size.
2. Electrotransfer (Western blotting) and revelation of the transferred proteins.
3. Immunodetection and quantification of Western stress protein expression.

The evaluation of the cytotoxicity of Cd and Mg "*in-vitro*" after exposing A549 pneumocytes to the latter is determined according to the following steps:

1. Cell count.
2. Determination of total proteins by the method of Lowry.
3. Quantification of the expression of the stress proteins on immunoblot.

Results

Search for the protective effects of mg vis-a-vis cd expression of Hsp 72/73 "*in vivo*"

At the level of the liver: Figure 1 shows that magnesium with the two doses used does not induce HSP expression in liver cells. On the other hand, the injection of cadmium chloride induces the expression of HSP 72/73. This is a highly significant induction. Simultaneous Mg injection did not induce significant over-expression of HSP 72/73 and did not significantly alter their cadmium-induced over-expression.

At the level of the testicles: The injection of Cd chloride induces the expression of HSP 72/73 significantly or even highly significant in the testes. The injection of Mg does not induce the expression of HSP 72/73 and does not significantly modify their expression by cadmium (Figure 2).

At the level of the kidneys: In the kidneys, Mg does not induce the expression of HSP 72/73. Injection of Cd chloride induces expression of HSP 72/73 at the renal cell level significantly from the first day of treatment. This induction is of the order of 30% only with respect to the controls (Figure 3), concerning the simultaneous injection of Mg, one notes the same result as for the liver and testicular cells.

In-vitro culture

Expression of Hsp 72: Figure 4 show that the addition of cadmium in the culture medium of A549 cells induces the expression of HSP 72. This induction is of the order of 60% compared to controls. Simultaneous injection of Mg does not significantly alter the over expression of Hsp 72 induced by cadmium. This "*in-vitro*" study confirms the results found "*in vivo*" in our experimental conditions.

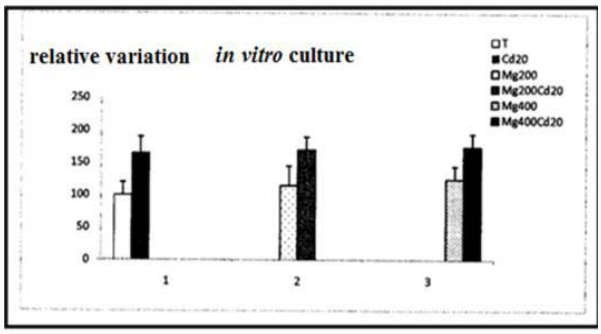

Figure 4: Expression Variation of the HSP72 Protein of A540 Cells Exposed to cadmium (Cd) with or without magnesium at different concentrations, (200 µM or 400 µM).

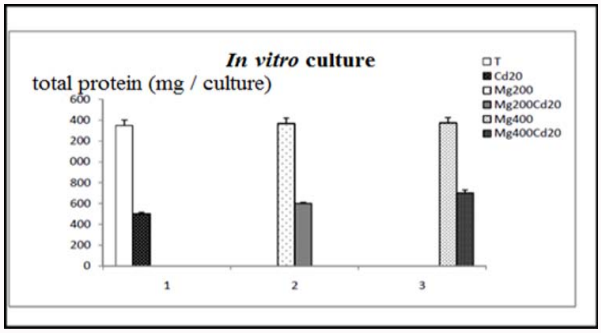

Figure 5: Effect of magnesium on cell proliferation, and/or the amount of total protein (µg)/culture of A540 cells.

Effect on cell proliferation and amount of total protein by culture: This study clearly shows the cytotoxic effect of Cd, and that the simultaneous addition of Mg to the culture medium of A549 cells at different concentrations, 200 µM and 400 µM does not diminish this effect (Figure 5). Therefore, it seems that if Mg is able to protect against the cytotoxic effects of Cd, at acceptable dose "*in vivo*" and this according to the previous chapter, it is not "*in-vitro*" at low and high doses which show that Mg seems to protect against the effects of Cd by directly interfering in this metal in terms of its absorption, transport and accumulation dynamics. A protective action of Mg at the level of defense and cellular repair systems seems to be rejected, given its inability to protect against the cytotoxic effects "*in-vitro*" at the doses used.

Discussion

All living cells have a system of adaptation to the environmental stress, once they are stressed by physical or chemical changes in their environment and react stereotypically: they stop doing what they usually do, their metabolism normal, to focus on the production of stress proteins, an immediate and transient production that allows the cell to adapt to the stress situation and survive. Therefore during a stress, the active principle of this product stimulates the synthesis and/or the activity of the physiologically active stress proteins (HSP) in the living organism, whose role is to oppose also the toxicity of the endogenous or exogenous free radicals produced by many molecules including oxygen. So the excess of R.L is a powerful stimulator of the synthesis of HSP. Stress proteins have the ability to repair other proteins, or eliminate them when they can no longer be repaired. They function as molecular chaperones, accompanying, monitoring and protecting other proteins. They participate in the folding of proteins, their subcellular transport and their translocation in intracellular organelles (mitochondria for example). So HSPs can also contribute to free radical protection. Examples of such protection are many both "*in-vivo*" and "*in-vitro*". It therefore appears that HSPs have the ability to intervene in antioxidant oxidative equilibrium, similar to other endogenous antioxidant systems. The HSP 70/72 but also the HSP 90, repair damaged proteins (unfolded) by stress, and stimulate the synthesis of active detoxification enzymes free radicals, indeed, they will oppose the destructive effects of the latter in repairing the depleted proteins, restoring them to functionality, including enzymes and in particular those of the antioxidant system. The most studied thermal shock proteins are HSC 70 and HSP 70. HSC is the constitutive protein of molecular weight around 70 KDa and HSP is the inducible protein. The regulation of the synthesis of HSP and HSC 70 involves transcription factors as well as other cellular constituents in eukaryotes, genes encoding heat shock proteins are under the control of Heat Shock Transcription Factors (HSF). These genes include a notifiable regulatory DNA called "Heat Shock Element" (HSE) which is notoriously repeated GAAn [3]. The latter has shown that induction of HSP expression is achieved when HSF binds to HSE thus forming a trimer. The transcriptional activity of HSF is also linked to phosphorylation [4], which is triggered by several stimuli such as MAP (Mitogen Activated Protein Kinases), which are induced by exposure of cells to free radicals. In fact, stress proteins are considered as markers of toxicity and their expression depends on the cytotoxicity of the dose and the duration of the treatment, and Wagner et al. [2] have shown that the increase in the expression of HSP 70 can be considered as a marker of the increase in stress resulting from environmental pollution. Thus, following an oxidative stress, the cell produces more HSP this overproduction is at the origin of the adaptation and cell resistance against reactive metabolites of oxygen. Our results show that Cd induces the expression of HSP 72/73, "*in-vivo*" which "*in-vitro*", this confirms the results of Almazan et al. [5]. Which showed that cadmium chloride induces a reduction in the concentration of intracellular glutathione, and a high accumulation of free radicals, thus causing cell death? Indeed, our *in-vitro* study showed the cytotoxic effect of Cd on A540 cells. Dunlap and Matsumara [1] have shown that HSP synthesis can increase in cultured cells by exposure to heavy metals. This confirms the results of Shelton et al. [6] which showed the induction of HSP 70 by the most toxic metals such as Cd, Pb and Hg Liu et al. [7] have shown that *in-vivo* Cd induces the expression of HSP 70 by the cells of the proximal tube and decreases their viability, as well as other studies have shown that a 4 hrs exposure of the cells of the proximal tube to the chloride of cadmium (53.4 µm) induces over-expression of HSP 60 and after 60 days there is cell death, but exposure to a lethal dose causes a decrease in the level of HSP 60 compared to controls. This shows a dual role for HSP 60, the first is protective when cells are currently exposed to Cd and a degenerative role when regulation is no longer maintained during prolonged or chronic exposure. Curtis et al. [8], have shown that the 14-day treatment of rats with Cd chloride (0.2 M) leads to a significant accumulation of this metal in the liver and the kidney, as well as an induction of HSP 72 by hepatic cells, which asserts the protective role of HSP 72 in Cd toxicity. The addition in the culture medium of hepatocyte cells of different concentrations of metals such as Cd and Zn induces over expression of HSP. (Bauman et al., 1993). Research studies have shown that Cd produced by industries representing a very important environmental pollutant can induce severe damage especially in the kidney where it accumulates. Barrouillet et al. [9] showed that Cd exhibits a dose-dependent cytotoxic effect on renal cells in culture. Indeed, an addition of Cd in the culture medium of glomerular cells, affects their growth with reduction of their size as a function of time thus indicating that this metal causes the contraction of the glomerular structure, which can explain the reduction of the filtration. Glomerular disease during Cd nephrotoxicity. The incorporation of Cd and Pb in the body alters the physiological processes of development. These two metals have a toxic effect on cell growth in addition they cause metabolic damage. Indeed, Cd significantly affects the maturation of Oocytes, causes their degeneration and decreases the percentage and viability of spermatozoa, so according to this study Leoni et al. [10] showed that "*in vitro*" to low doses, Cd affects the physiological function of gametes but at high doses, it affects cell viability. Xu et al. [11], have shown that "*in-vivo*" cadmium chloride induces fragmentation of rat testicular DNA, thereby causing apoptosis. Indeed, the culture

of lung epithelial cells in the presence of Cd, shows that the latter causes a morphological alteration characteristic of the phenomenon of apoptosis. The changes consist in the fact that the cells are detached from their neighbors and the condensation of cytoplasma and chromatin [12]. So the exposure of these cells to Cd, leads to an increase in the rate of HSP 72/73. The *in-vitro* culture of monocytes, the most sensitive leukocytes for the toxic effect of Cd [13], leads to cell death depending on the concentration and duration of treatment. According to the same study, Steffensen et al. [14] have also shown that Cd causes serious destruction of the cell membrane; in fact its cytotoxic effects are related to its accumulation in the cell. Injection of magnesium sulfate, or the addition of Mg, does not induce the expression of HSP 72/73. This confirms the results of Gilland et al. [15] who have shown that the injection of Mg sulfate into adult rats does not induce the expression of HSP 72. Therefore Mg seems to protect against the effects of Cd in interfering directly on this metal in terms of its absorption, transport and accumulation dynamics.

Conclusion

Our results show that treatment with Cd chloride induces over-expression of HSP 72/73 in the liver, testes and kidneys. The single or simultaneous injection of Mg does not induce the expression of these proteins and does not modify their over-expression induced by Cd. This over-expression serves to protect the body against Cd, indeed it is considered as a biomarker of exposure. The culture "*in-vitro*" confirms these results where the addition of Cd in the culture medium of A549 cells induces an over expression of HSP 72 and the simultaneous addition of Mg does not modify the latter. The same study showed the cytotoxic effect of Cd and that Mg does not decrease this effect inversely to what has been shown in our previous studies in which the protective effects of Mg against the cytotoxic effects of Cd were shown *in vivo*. The protective effect of Mg appears to be to interact with the absorption, transport and accumulation dynamics of Cd in the body, which lowers or lowers its endogenous exposure levels and consequently its toxic effects.

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