The protective effect of Mg on accumulation biomarkers and markers of Cd-induced oxidative stress in adult male wistar rats

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Abstract

The treatment with cadmium chloride results in a significant accumulation of cadmium in the liver, kidneys and testes accompanied by an increase of the level of MDA with the already chosen organs in treated animals, testifying a lipid peroxidation. A decrease of serum levels of vitamins E and A, which testifies the ability of Cd to induce free radicals (FR), hence the oxidative power of this metal. The induction of SOD activity at the beginning of treatment could also be explained by the production of the FR an inhibition of SOD activity at the end of the treatment. It could also be due to an excessive production of FR in the end treatment. The treatment with magnesium sulphate results in decreased Cd accumulation in the liver, kidneys and testes, and a lower increase in the liver, kidney and testicular MDA levels. The Mg limits the fall in vitamins E and A in Cd-treated rats and Cd-induced oxidative stress since SOD activity is less affected.

Keywords: cadmium; magnesium; free radicals; oxidative stress; antioxidant enzymes; lipid peroxidation.

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Introduction

Several studies have shown that the disturbances caused by Cd are mainly due to its cytotoxic Effects inducing peroxidation phenomena. This metal is therefore classified as oxidative stress and, therefore, it is an exogenous source of free radicals (FR), which can generate a significant tissue and membrane damage in the body. However, low-dose magnesium could play the role of an antioxidant according to several literature reviews [1, 2]. In this context, we proposed to explore

The biomarkers of exposure of Cd in the body of a male adult wistar rat (liver, kidneys and testes). The impact of Mg on biomarkers of Cd accumulation at the level of the already chosen organs. The interaction between Mg and the oxidative effects of Cd through the dosages of MDA (Malone dialdehyde) and through the exploration of myeloperoxidase (MPO). In this context, we have attempted to explore in the male adult wistar rat:
The Bio-markers of Cd exposure in the body (liver, kidneys and testes).

The impact of the Mg on the biomarkers of the Cd accumulation at the level of the already selected bodies).

The interaction between the Mg and the oxidative effects of the Cd through both the Dosages of the MDA (Malone dialdehyde) and exploration of the myeloperoxidase (MPO).

Therefore, a solution of chloride of Cd (2, 5 mg/kg), enriched in magnesium sulfate or not (300 and 600 mg /body weight) was intra-peritoneal injected into male rats during 0,1,5 and 10 days.

Material and methods

Ethics Statement

The Ethics Committee of the Faculty of Sciences of Sfax approved the experimental protocol used in the present study. The guidelines established by this Faculty to take care and use of laboratory animals were followed in all experimental procedures.

Animals

Male Wistar rats weighing between 150 and 180 g were obtained from the animal house of Sfax University, Tunisia. Animals were kept under the following conditions: temperature (25±2°C), humidity (60±10%), a 12/12 h light-dark cycle and allowed to access water and food freely.

Chemicals

Cadmium, magnesium and solvents were purchased from Sigma-Aldrich.

Experimental design

This protocol is carried out 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 body weight (Mg1 group) and 600 mg / kg body weight (group Mg2). The next day, noted jo, rats that are injected with NaCl are divided into two groups, one was injected throughout the experimental period by the physiological liquid qualifies as a control (T group), the other through chloride cadmium at 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 thereto. The treatment lasts between 0.1, 5 and 10 days.

Estimation of lipid peroxidation

The level of lipid peroxidation in the animal tissues was estimated by measuring thiobarbituric acid reactive species (TBARS) Yagi according to Yagi (1976) [3]. About 1g of rat organs, namely the liver and kidneys, was cut into small pieces and immersed into 2 ml ice-cold lysis Tris-buffered saline (TBS, ph 7.4). The mixture was then sonicated (twice for 10 seconds,) and centrifuged (5000g, 30 min, 4°C). Supernatants were collected and stored at – 80°C until use. For the assay, 125 µl of supernatants were homogenized by sonication with 50 µl of TBS, 125 µl of TCA-BHT in order to precipitate proteins and centrifuged (1000g, 10 min, 4°C). 200 µl of the resulting supernatant were mixed with 40µl of HCl (0.6M) and 160 µl of TBA (dissolved in Tris), and the mixture heated at 80°C for 10 minutes. The absorbance of the supernatant was then read at 530 nm. The amount of 2-thiobarbituric acid-reactive material (TBA-rm) was calculated using an extinction coefficient of 156 mm-1 cm-1.

Determination of the cadmium rate

Kidneys, testes and liver dehydrated at 80 °C are mineralized in the liquid phase by the nitroperchloric acid attack method 2V / 1V. Thus 1g of dry matter (kidney, testicle and liver) is put in a kjeldhal flask to which 5ml of nitric acid and 2.5ml of acid are added. Perchloric. The matras are then placed on the digestion ramp at an average temperature of (200°C). The end of the mineralization is characterized by a release of white smoke. After being cooled, the content of the flask (mineralized) is filtered and the volume is adjusted to 5 ml by distilled water. A white sample is prepared in the same way but with 1 ml of distilled water. The cadmium assay is carried out in the Environmental Science Laboratory (LARSEN – ENIS Sfax) using a Zeeman Z 61000 atomic absorption spectrophotometer (HITA CHI brand) at a wavelength of 23, 5 nm.

Determination of the myeloperoxidase (MPO)

In this study, we used a KIT Immunotech (réf. 1119) for staining with Alphanaphthol-pyronine. Myeloperoxidase
granulations form a bright red insoluble complex with pyroxin in the presence of alphanophtol and hydrogen peroxide [4]. The leucocyte nuclei were stained blue by Mayer's hematoxylin [5].

**Histological analyses**

Classical procedures were used for histology. After fixation in Bouin solution, pieces of fixed tissue were embedded into paraffin, cut into 5µm slices and colored with hematoxyline-eosine.

**Statistical analysis**

Two independent experiments were performed. Data were expressed as mean ± standard deviation (SD). Statistical significance was assessed by ANNOVA test. *P < 0.05 was considered statistically significant.

**Results**

**Variation of the Cd rate in the liver**

Our results show that, on the one hand, there is a very large and highly significant increase of the Cd level in the liver of the rats injected with only Cd compared to controls (T) during 5 and 10 days and, on the other hand, this accumulation significantly decreases by using Mg which is still of a dose-dependent nature mainly during the fifth day. (figure1).

![Figure 1](image)

**Figure 1:** Liver content of Cd: (µg / g dry weight) of control rats (T) and rats injected with cadmium chloride (Cd) alone or in combination with Magnesium (CdMg₂) and (CdMg₂). Values represent the average ± SD (n= 10).

**: P ≤ 0,01 by compared with the control rats.

**: + P ≤ 0,01 compared with the injected rats (Cd).

**: + P ≤ 0,05 compared with the injected rats (Cd).

**: - P ≤ 0, compared with the injected rats (Cd Mg1).

**Change of the Cd level in the kidneys:**

The (figure 2) shows that the injection of Cd and Cd associated with the two doses of Mg causes a highly significant increase in the Cd content of Cd rats; Cd Mg1 and Cd Mg2 compared to controls. This accumulation decreases in a highly significant and dose-dependent manner by associating Mg with Cd with respect to Cd alone.

**Variation of the Cd level in the testicles**

Our results show a highly significant increase in Cd testes content for different groups of Cd injected rats; Cd Mg1 and Mg2 with respect to T during 5 and 10D. Similarly, there is a decrease in this accumulation in a significant or highly significant manner by associating the Mg with the Cd with respect to the Cd alone. This decrease of Cd accumulation is dose-dependent (Figure 3).

**Variation in hepatic MDA or hepatic TBARS**

Figure (4) shows that the injection of Mg alone during the day before the treatment with CdCl₂ leads to a decrease of the level of hepatic MDA in the treated rats compared to the controls. Being of a dose-dependent nature, this reduction is more important with Mg2 than with Mg1.
Figure 2: Kidney content of Cd: (μg / g dry weight) of control rats (T) and rats injected with cadmium chloride (Cd) alone or in combination with magnesium (CdMg₁) and (CdMg₂). Values represent the average ± SD (n= 10).

** : P ≤ 0,01 compared with the control rats.
++ : P ≤ 0,01 compared with the injected rats. (Cd)
- : P ≤ 0,05 compared with the injected rats (Cd Mg₁).
-- : P ≤ 0,01 compared with the injected rats (Cd Mg₁).

Figure 3: Testicle content of Cd (μg / g dry weight) of control rats (T) and rats injected with cadmium chloride (Cd) alone or in combination with magnesium (CdMg₁) and (CdMg₂). Values represent the average ± SD (n= 10).

** : P ≤ 0,01 compared with the control rats.
* : P ≤ 0,05 compared with the control rats
++ : P ≤ 0,01 compared with the injected rats. (Cd)
+ : P ≤ 0,05 compared with the injected rats. (Cd)
- : P ≤ 0,05 par compared with the injected rats (Cd Mg₁).

However, the injection of Cd for 1.5 and 10 days increases this rate in a highly significant manner. This increase is antagonized by a simultaneous injection of Mg. The basic rate of the MDA, which is relatively high among the controls on day 0, can be explained by the stressful effect of the needle during the injection.
Figure 4: MDA (nmol / mg) level in the testicles of control rats (T) and rats injected with cadmium chloride (Cd) alone or in combination with magnesium (CdMg₂) and (CdMg₄)

Values represent the average ± SD (n= 10).

** : P ≤ 0,01 compared with the control rats.

* : P ≤ 0,05 compared with the control rats.

++ : P ≤ 0,01 compared with the injected rats. (Cd)

--- : P ≤ 0,01 compared with the injected rats. (Cd Mg1)

**Variation of the renal MDA rate or renal TBARS**

The cadmic poisoning significantly increases the MDA rate after 1, 5, and 10 days of treatment compared to the controls. However, a simultaneous injection of magnesium sulfate significantly reduces, with a dose-dependent nature, the effect of the Cd lipoperoxydatif (Figures 1, 2).

**Variation of the testicular MDA rate or the testicular TBARQ**

Our results showed that the injection of the Cd leads to a lipid peroxidation at the level of the testes. Moreover, the induction of the latter is highly significant compared to the controls. In fact, for the rats simultaneously injected with the mgso4, there is a decrease of this induction (CdMg₂; CD Mg₂) which is still of a dose-dependent nature.

**Demonstration of myeloperoxidase (MPO)**

Our results showed that the leukocyte nuclei of the controls are stained blue (Figure5), whereas those of the CdCl₂ treated rats are red (slightly pink) (Figure 6), which indicates the presence of myeloperoxidase, and therefore of free radicals (ROS). Then, the association of Mg with Cd limits the release of these radicals (Figure 7). Indeed, the MPO is a specific reflection of neutrophil activation and an indirect generation of the ROS.

Figure 5: Leukocytes from control rats (blue nuclei).

**Impact on the level of serum vitamin E**

Vitamin E reacts with free radicals to form the radical tocopleryle, a stable substance the effect of which is to stop the chain reactions caused by the free radicals. Our results about the dosage of vitamin E (tocopherol) at the serum level show that the injection of cadmium causes a significant decrease
during the 5th day (figure 8), which implies the formation of free radicals (FR) caused by the Cd as well as the depletion of vitamin E. The simultaneous injection of magnesium sulfate significantly increases the rate of vitamin E with a dose-dependent nature compared to rats treated with Cd only, besides, the values found were almost the control ones (normal state). This confirms the protective role of magnesium in protecting the body from the cadmium oxidizing effects.

**Impact on the level of serum vitamin A**

Actually, Vitamin A reacts with singlet oxygen and can thus prevent the oxidation of several organic substrates, such as polyunsaturated fatty acids. Figure (9) shows that the Cd treatment causes a highly significant reduction of the level of vitamin A, which implies the presence of active oxygenated species (ROS) induced by the Cd as well as the exhaustion of vitamin A.

A simultaneous injection with the Mg, prevents this reduction. Hence, the Cd acts as a stressor with an oxidative effect while the intervention of an antioxidant, such as the Mg, protects against this effect.

**Impact on the activity of the erythrocytic SOD**

Our results show that injection with the Cd causes an increase of the SOD activity during the 1st day, then, there is an inhibition for the rest of the treatment (figure 10).

![Figure 6: Demonstration of myeloperoxidase: Leucocytes in Cd-treated rats (red nuclei).](image)

![Figure 7: Demonstration of myeloperoxidase: Leukocytes from rats treated with Mg-associated Cd.](image)
**Figure 8:** Level of vitamin E (μg / ml) control rats (T) and rats injected with cadmium chloride (Cd) alone or in combination with magnesium (CdMg₁) and (CdMg₂).

The values represent the mean ± ESM (n = 10).

****: P ≤ 0.01 compared to the control rats.

*: P ≤ 0.05 compared with the control rats.

++: P ≤ 0.01 compared with the injected rats (Cd).

+: P ≤ 0.05 compared to the injected rats (Cd).

**Figure 9:** Level of the vitamin A (μg / ml) control rats (T) and rats injected with cadmium chloride (Cd) alone or in combination with magnesium (CdMg₁) and (CdMg₂).

The values represent the mean ± ESM (n = 10).

**: P ≤ 0.01 compared to the control rats.

+: P ≤ 0.05 compared to the injected rats (Cd).

++: P ≤ 0.01 compared with the injected rats (Cd).
**Discussion**

If it is vital for our organism, the $\text{O}_2$ could also be one of the causes of its degradation since it is at the origin of very reactive derivatives thus unstable, it is the reactive species of $\text{O}_2$ (ROS) called more generally free radicals. Three endogenous ROS generation pathways are generally described, namely: the electron transfer chain located at the level of mitochondria [6]; phagocytic cells, the latter being essential for immune defense; as well as the activity of oxidase enzymes [7]. Other sources or modes of exogenous generation of the ROS exist through interactions with their surrounding environment, for example, the ingestion or inhalation of toxic, oxidized or oxidizing products [8].

These sources are mainly environmental pro-oxidants, such as pesticides, heavy metals (Cd, Pb, Ni ...), cigarette smoke, pollutants that can produce oxidative stress. The latter is increasingly studied both in the field of research and in human medicine. It has been defined as an imbalance between antioxidant and oxidizing elements in favor of the latter and their potentially harmful effects. Its origins are multiple and result from a formation of the ROS within the organization. In fact, oxidative stress (or oxidative pressure) is a type of aggression of the constituents of the cell due to the ROS. The difficulty of directly demonstrating oxidative stress is that the ROS have an extremely short half-life. The techniques used to dose the ROS are complex, expensive and difficult to achieve in practice. Consequently, products resulting from the oxidation processes called oxidative markers are used to indirectly highlight and quantify an oxidative stress suffered by the organization. In addition, antioxidants can be dosed to highlight any shortcomings or an imbalance between the anti and pro oxidants. Since Cd is classified as a pro-oxidant and the important role of bio-markers in detecting, preventing and treating the early and reversible effect of environmental intoxication, Cd levels in the liver, kidneys and testes and its oxidative effects by measuring the level of MDA or TBARS (lipoperoxidation products) in the three organs already mentioned and by the detection or the presence of myeloperoxidase (MPO). Our results showed that the injection of cadmium chloride ($\text{CdCl}_2$) during 5 and 10 days, a highly significant accumulation in the three already chosen organs (liver, kidneys, testes) with high levels are found in the liver and kidneys and that the injection of mgso4 decreases the latter in a significant or highly significant way even it is of a dose-dependent nature. In fact, bibliographic studies have shown that Cd is effectively retained in the kidneys and liver, with a half-life of 20 to 30 years. It can cause the demineralization of the bones, by direct damage as a consequence of renal dysfunction as it has been shown in the 1st chapter where there is evidence of renal failure following a chronic exposure to Cd chloride.
The Cd is actually chosen for two reasons, first of all, it is one of the most toxic and abundant elements in the waste, besides, it is responsible for serious intoxications in the bones, lungs, digestive tract and kidneys. Our study also showed that the injection of Cd chloride (2.5 mg / kg Cd) resulted in an increase of the level of lipid peroxidation in the three organs during the first day of the injection. This shows the attack of lipid membranes by RL and the peroxidative effect of Cd. This again is certainly related to the high Cd contents of these three organs already treated. Since Cd accumulation is mainly in the kidneys, this organ is considered a "target" organ and therefore a chronic exposure to Cd leads to the appearance of irreversible nephropathy that may cause kidney failure. Other researchers, such as [9] that Cd accumulates mainly in the kidneys, liver, testes and lungs. Similarly, COMPANA et al (2003) showed that under the effect of the accumulation of metals in the kidneys and liver, the lysosomes of these organs will be activated thus inducing oxidative stress. Some studies [10] pointed out to the involvement of heavy metals in the genesis of the ROS responsible for oxidative damage. These changes could pose a great risk for reproduction (already noted peroxidative effect of Cd in the testes) and the survival of the organisms exposed to risk [11]. Similarly, [12, 13] showed that in the cell, several effects of Cd are noted by an increase of the production of reactive species or forms of oxygen (ROS) associated with a reduction of the glutathione levels [14-16]. In fact, the ROS can potentially react with each cell component and then cause its oxidation. However, the preferential targets of these species are particularly the membrane lipid structures, where they attack the membranes that surround and protect the cells of the body. They then destabilize these structures, accelerate the deterioration of cells and tissues. Liperoxides that escape detoxification lead to toxic aldehydes, the best known is the malondialdehyde (MDA) resulting from the cleavage, induced by free radicals polyunsaturated fatty acids having at least three double bonds. Similarly, Uyesaka et al., 1992; OOSTENBERG et al., 1997 showed that the oxidation of these cellular components can induce dysfunctions of cellular metabolism, such as a change in the membrane fluidity caused by lipid peroxidation or a decrease of the activity of an enzyme due to its oxidation [17].

In the same context, other research has also shown the ability of Cd to increase the rate of lipid peroxidation DNA damage and proteins [18]. Measuring MDA is therefore an excellent marker for the study of the effect of toxic substances and the assessment of their potential toxicity.

The excessive production of MDA recorded in this study is certainly a significant fraction of the prooxidant action of cadmium. In addition, the role of this metal in the over production of MDA has been reported by some authors [10, 19-21].

Indeed, other researchers showed that treatment with Cd leads to an increase of MDA in the liver, kidneys and testicles. The increase in of MDA at the level of the testicles confirms the oxidative effect of Cd, which plays an important role in the sperm alteration process. The FR can thus modify the membrane structure as well as that of deoxyribonucleic acid. In fact, male infertility is a big problem. Several factors are at the origin of this phenomenon. Currently, oxidative stress is incriminated as one of the main causes. In fact, there is a correlation between the markers of oxidative stress and spermatic characteristics (count, motility) as they were shown in the 1st chapter where there is a decrease of in the number and motility of the spermatozoa of rats treated with cadmium. The effects of this metal are multiple and complex and the intracellular damage generated concerns all types of bio-molecules: denaturing of some proteins, lipid peroxidation, DNA breakage, repression or, on the contrary, over-expression of many genes [22, 23]. Similarly showed that oxidative stress will denature lipids, proteins, DNA and cause pathologies. In fact, lipid peroxidation is followed by a structural change in biological membranes [24,25] or other lipid-containing elements [26]. There will be a loss of membrane permeability and potential as well as the inactivation of receptors and membrane enzymes. These functional disturbances can lead to cell death as it was shown in the first chapter where the phenomenon of apoptosis caused by cadmium treatment was demonstrated.
Indeed, the treatment with the antioxidant, such as ascorbic acid, vitamins A, E, C and magnesium decreases the effect and protects the animals against the oxidizing effect of a heavy metal [2]. Thus, magnesium (Mg), which is a macro-element or mineral salt with amazing properties well known as anti stress, is essential for the proper functioning of our body. Research suggests that a deficit in Mg would promote atherosclerosis, dystlipidemia, which induces an inflammatory response. Conversely, it has been shown that Mg decreases inflammation, oxidative stress where it prevents the RL from entering the cells. Therefore, our results show that treatment with two-dose Mg 300 and 600 mg / kg significantly reduces the level of MDA in rats treated with Cd. This decrease is of a dose-dependent nature in the three organs (kidneys, liver and testicules). Mg supplementation decreases the level of MDA and its deficiency increases oxidative stress [1]. YASAR showed that copper supplementation protects against cadmium-induced oxidative effects in the liver, kidneys and placental tissues of pregnant rats and the fetus. He actually noted that the MDA rate and myeloperoxidase (MPO) activity are lower than in the case of cadmic exposure alone. Similarly MATOVIC, 2011, showed that an addition of Mg protects during exposure to Cd, hence the beneficial effect of supplementing essential elements, such as Mg and Zn against the oxidative power of Cd which has toxic effects on the kidneys and sexual organs. This is also in agreement with the work of [27] where they showed that Cd can cause dysfunctions of various organs and that a supplementation Mg can protect against the toxic effects of this metal thanks to its antioxidant properties. Similarly, showed that supplementation of Mg reduces the levels of the Cd compared to those of animals treated with Cd only. Indeed, the accumulation of Cd in the body is closed with supplements. Of Mg, Vit C. Other research [28] also showed that Cd accumulation in the kidneys and liver can be reduced by following a diet rich in polysaccharide extracted from crustacean meat. Many markers of oxidative stress are still to be developed and studied, although for some, the analysis techniques, such as those developed for the myeloperoxidase, Oxidized DNA [29], are now advanced. According to the amount of MDA and to the above considerations, the oxidative effect of Cd has been shown by inducing lipid peroxidation causing the release of neutrophils which, once activated, increase their consumption of O2 and generate a massive release of proteolytic enzymes. Moreover, lipid peroxidation is considered as a specific reflection of neutrophil activation and an indirect generation of the ROS.

Therefore, the detection of the myeloperoxidase or the presence of this enzyme is a marker of White cells activation and an indirect indicator of the presence of the ROS [30] and consequently a second confirmation of the oxidative power of Cd. Thus causing oxidative stress. In fact, according to this study, it has been shown that Cd behaves as a stressor with an oxidative effect and the intervention of an antioxidant, such as magnesium, decreases this effect, so the oxidative effect of Cd could be at the origin of its pathological effects on the body by affecting the kidney function and male and female fertility.

**Conclusion**

**Our results show that in adult male rats:**

* Treatment with cadmium chloride leads to a significant accumulation of cadmium in the liver, kidneys and testes.

* An increase of the level of MDA in the liver, kidneys and testes in the treated animals, which shows the peroxidative effect of metal on the three organs.

* The demonstration of the presence of myeloperoxidase. Indeed, the latter is an indirect indicator of the presence of the ROS and therefore the powerful oxidizing of cadmium causing oxidative stress.

Treatment with magnesium sulfate associated with Cd leads to:

* A decreased Cd accumulation in the liver, kidneys and testes of a dose-dependent nature

* A slight increase of the hepatic, renal and testicular MDA levels only cadmium alone, which implies the protective effect of Mg against Cd cytotoxicity.

* A decrease of the MPO release In fact, cadmium toxicity is related to its accumulation in the body.

**Abbreviations:** MPO: myeloperoxidase, Cd: cadmium, Mg: magnesium, FR: free radicals, SOD: antioxidants, TBARS: superoxide dismutase, thiobarbutiric: acid reactive species.
Conflict of Interest
The authors declare no conflict of interest. The founding sponsors have not been involved at any stage of the study. They participated neither in the design and the conduction of the experiment, nor, in the analysis of the data or the preparation of the manuscript for publication.

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