Protective role of vitamin E against cadmium induced oxidative stress into the rat liver

Rôle protecteur de la vitamine E contre le stress oxydatif induit par le cadmium dans le foie du rat

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RÉSUMÉ

Introduction : Le Cadmium (Cd) est un métal lourd toxique utilisé dans plusieurs applications industrielles et peut causer, suite à l'exposition environnementale ou professionnelle , plusieurs dégâts dans tous les systèmes du corps. Notre étude a été élaborée afin de déterminer l'effet toxique d'une dose élevée du Cd sur le foie du rat ainsi que l'effet protecteur putatif de la vitamine E.
Méthodes: Durant cette expérience, le Cd a été administré par voie orale (PO) (15 mg / kg du poids corporel) seul ou associé à une injection intra-péritonéale (IP) d'alpha-tocophérol (vitamine E) (300 mg / kg / jour) pendant trois semaines. Nous avons analysé l'effet de la vitamine E sur des fragments prélevés du foie des rats traités par le Cd et colorés à l'hématoxyline-éosine en vue d'une étude anatomopathologique, ainsi que par la détermination des profils antioxydants et la peroxydation des lipides .
Résultats: Les données ont confirmé que la dose élevée du Cd induit une perte du poids du foie et un état pro-oxydant dans les hépatocytes caractérisés par une augmentation du taux du malondialdéhyde (MDA) et de la peroxydase (POD), aucun changement du taux de la catalase (CAT) et une diminution de l' activité de la superoxyde dismutase (SOD). Ces perturbations peuvent être expliquées par une diminution du taux du calcium hépatique (Ca). Le co-traitement avec la vitamine E, induit une diminution du taux du MDA et de POD, une augmentation des activités de la CAT et de la SOD, et une restauration du taux du Ca. Toutes ces corrections ont été accompagnées par une amélioration de la structure du foie.
Conclusion: Nos résultats suggèrent que le Cd induit un stress oxydatif dans le foie du rat et que la vitamine E exerce des propriétés antioxydantes qui peuvent être médiées par la modulation du niveau du Ca.

Mots-clés
cadmium; stress oxydatif ; activités antioxydantes; vitamine E; Calcium

SUMMARY

Introduction : Cadmium (Cd) is a toxic heavy metal used in various industrial applications and therefore can cause, both by environmental or professional exposure, several damage in all body systems. The present study was developed to determine the toxic effect of high dose of Cd on the rat's liver as well as the putative protective effect of vitamin E.
Methods: During the experiment, rats were administrated Cd per orally (PO) (15mg/Kg bw) alone or associated with an intraperitonial (IP) injection of alphatocopherol (Vitamin E) (300mg/Kg / day) for three weeks. We analyzed the effect of vitamin E on Cd induced liver remodeling by hematoxylin-eosin staining (HE), and by the determination of the antioxidant profiles and lipid peroxidation in rats's livers.
Results: Data confirmed that high dose of cd induced a loss of the liver weight and a pro-oxidative state into hepatocytes characterized by increased malondialdehyde (MDA) and peroxidase (POD), no changes in catalase (CAT) and a decrease on the superoxide dismutase (SOD) activities. These disturbances may be explained by a decrease in the level of hepatic calcium (Ca). Co-treatment with Vitamin E, decreased MDA and POD activities, increased CAT and SOD activities and restored Ca level. All these corrections were accompanied by an improvement of the liver 's structure.
Conclusion: Our results suggest that Cd induced an oxidative stress into rat liver and Vitamin E exerted antioxidant properties which can be mediated by the modulation of Ca level.

Key-words
Cadmium ; oxidative stress ; antioxidant activities ; Vitamin E ; Calcium.

INTRODUCTION

Cadmium is a heavy metal found in the earth's crust and released into the environment by both natural processes and human activities such as burning of fossil fuels, waste incineration and the use of phosphate fertilizers [1].

Cadmium can be absorbed and accumulated in plants and animals through water, air and soil, and hence in the human body through the food chain [2]. Indeed, food contaminated with cadmium is a major source of human exposure(fish, meat, cereals, crustaceans bodies mainly the liver and kidney) [3]. This toxic metal have a biological half-life of extremely long15 to 20 years in humans [4] which can be spread in some subjects up to 30 years [5] allowing it to be accumulated slowly in living cells causing adverse effects on the human health. This prompted us to examine putative protective role of Vitamin E that may be offered against the toxic effect of cadmium in food. Vitamin E has some important molecular properties such as the capture of Reactive oxygen species (ROS) and the modulation of signal transduction and gene expression by acting as an antioxidant [6]. It is a scavenger of peroxyl radicals (ROO•), preventing their spread within the membranes [7].

The present study was developed to determine the toxic effect of Cd on rat liver as well as the putative protection that may be offered by Vitamin E.

METHODS

Animals and treatments

In this experiment, 24 male Wistar rats (180-200g) were maintained in animal house at fixed temperature of 22±2°C with 12h light-dark cycle. They were provided with water and food *ad libitum.* All experiments were performed according to the recommendations of the ethic committee of Tunis University for care and use of animals in conformity with NIH guidelines.

Chemicals

2-thiobarbituric acid (TBA); 2,6,-di-tert-butyl-4-hydroxytoluene (BHT); trichloroacetic acid (TCA); hydrogen peroxide(H_2O_2); 2-methoxyphenol (gaiacol); bovine catalase, 4-(1-hydroxy-2-methylamino-ethyl)-benzene-1,2-diol (epinephrine) and 2,4,dinitrophenyl hydrazine (DNPH) were obtained from Sigma-Aldrich (Germany).

The experimental Groups

Rats were randomly divided into 3 groups of 8 animals each. Group 1 of rats received a standard diet, group 2 treated with Cd (15 mg Cd /Kg *bw* in food) and group 3 treated with Cd (15mgCd /Kg *bw* in food) + vitamin E (300mg /kg *bw*/ day) as alphatocopherol acetate administered by intraperitoneal injection .After treatment (21 days), rats were anesthetized with urethane (40mg/ml), the livers collected was weighed using a precision balance (0.001) and pieces of these organs were placed in the formaldehyde for an histological study. Other pieces of these organs were weighed, homogenized in PBS buffer (pH: 7,4) with an ultrathorax T25 homogenisator and centrifuged (10 min at 10000 g, 4° C) .

The supernatants were aliquoted and stored at -20°Cand then used for the determination of the levels of antioxidant enzymes (SOD, catalase, peroxidases) and for the measurement of indicators of oxidative stress and / or cellular death (MDA, Ca^{2+}).

Lipid peroxidation

Lipid peroxidation was assessed by MDA measurement according to the double heating method [8]. Briefly, aliquots (200 µL) from liver homogenates were mixed with 250µL BHT-TCA solution containing 1% BHT (m/v) dissolved in 20% TCA (m/v) and centrifuged at 1000 g for 5 min at 4°C. The supernatant (400 µL) was blended with 0,5N HCl (80 µl), and 120 mM TBA in 26 mM Tris (320 µL) and then heated at 80°C for 10 min. After cooling, absorbance of the resulting pink chromophore was determined at 532 nm using a SmartSpec 3000 Bio-Rad UV-visible spectrophotometer (Germany). MDA levels were determined by using an extinction coefficient for MDATBA complex of 1,56 10^5 M^{-1} cm^{-1}.

Calcium measurement

The quantities of calcium in the liver was determined according to Stern and Lewis [9] using a commercially available kit from Biomaghreb, Tunisia. At basic pH, calcium constitutes with cresolphtalein a purple colorful complex measurable at 570 nm. Briefly, 50 µL of the supernatants was added to 650 µL of reaction mixture containing 2-amino-2-methyl 1-propanol buffer (500 $mmol.L^{-1}$), cresolphtalein (0.62 $mmol.L^{-1}$) and hydroxy-8 quinoleine (69 $mmol.L^{-1}$). Incubation was carried out at room temperature for 5 min assuming the complex was stable for one hour.

Protein determination

Total soluble proteins were determined according to the biuret method [10]. Briefly, at acidic pH, soluble proteins constituted with copper a colorful complex measurable at 546 nm using a SmartSpec 3000 Bio-Rad UV-visible spectrophotometer.

Antioxidant enzyme activity assays

All spectrophotometric analyses of antioxidant enzyme activities were performed with a SmartSpec 3000 Bio-Rad UV visible spectrophotometer.

Catalase: Catalase (CAT) activity was determined by measuring the initial rate of H_2O_2 disappearance at 240 nm [11]. The reaction mixture contained 33 mM (1000 µL) H_2O_2in 50 mM (1995 µL) phosphate buffer (pH 7.0) and 5 µL of the supernatant. CAT activity was calculated using an extinction coefficient of 40 mM^{-1} cm^{-1} for H_2O_2.

Peroxidase: Peroxidase (POD) activity was measured at 25°C using guaiacol as hydrogen donor. The reaction mixture contained 9 mM (25 µL) guaiacol, 19 mM (100 µL) H_2O_2 in 50 mM (870µL) phosphate buffer pH 7 and 5 µL of the supernatant in a final volume of 1 mL. The reaction was initiated by the addition of H_2O_2 and monitored by measuring the increase in absorbance at 470 nm every 30 s for 3 min. POD activity was expressed as nmol of guaiacol oxidized per min and calculated using a molecular extinction coefficient of 26,2 mM^{-1}.

Superoxide dismutase: Superoxide dismutase (SOD) activity was determined by using a modified epinephrine assay [12]. At alkaline pH, superoxide anion (O^{2-}) causes the auto-oxidation of epinephrine to adenochrome. One unit of SOD is defined as the amount of extract that inhibits the rate of adenochrome formation by 50%. The supernatant was added to a 2 mL reaction mixture containing 10 µL bovine catalase (0,4 $U.\mu L^{-1}$), 20 µL epinephrine (5 $mg.mL^{-1}$) and 62,5 mM sodium bicarbonate buffer (pH 10,2). Changes in absorbance were recorded at 480 nm.`

Histological study

For histological studies, a portion of the liver were perfused and immersed with fixative solution (10% neutral-buffered formalin), for 24 h, and the blocks were taken thereafter. Tissue blocks were placed in formalin dehydrated in a graded series of ethanol, embedded in paraffin, cut into 4 mm thick serial sections, and stained with HE.

Statistical analysis

Statistical analysis of the data was performed by using Student's t-test and ANOVA, followed by Bonferroni's test. Significance was considered for p ‹ 0, 05.

RESULTS

Effect of Cadmium and Vitamin E on liver weight

Following the treatment of rats with a high dose of Cd (15mg /kg *bw*),there has been a dramatic decrease in the liver weight of approximately 4 grams compared to the control group. Treatment with Vitamin E counteracted this decrease.

Effect of Cadmium and Vitamin E on lipid peroxidation in the liver

Treatment with a high dose of Cd induced an increase of MDA level in the liver, this reflects the increase on lipid peroxidation induced by cadmium in this organ. Treatment with vitamin E abrogated the pro-oxidative effect of Cd to nearly 50% in this rate.

Effect of Cadmium and Vitamin E on liver antioxidant activities

The rate of catalase in the liver is kept steady by the treatment with Cd. However, an increase was observed in this rate compared to the control group after treatment with vitamin E (Fig3A). On the other hand, the rate of SOD in the liver was decreased when compared to control. Co-treatment with Cd and vitamin E conteracted all these effects (Fig 3B). Finally, the rate of total peroxidases increased twice after treatment with cadmium in comparison with control. Vitamin E abrogated induced Cd increase to control level (Fig 3C).

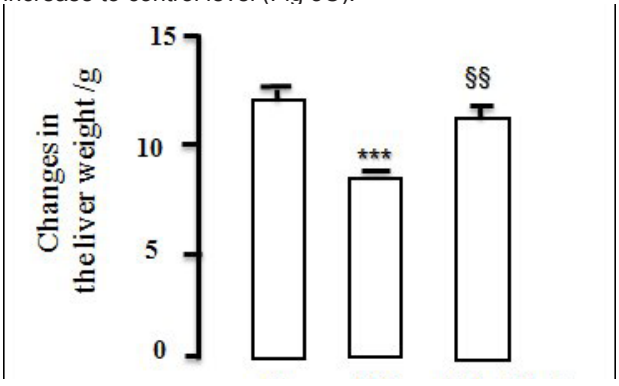

Figure 1: Effect of Cadmium and Vitamin E on liver weight. Rats were daily administered with 10% ethanol (C), Cadmium (Cd), or Cadmium and Vitamin E (Cd/vitE) for 21days. Results are expressed by means ± S.E.M. (n=8); ***$p<0,001$ *vs. C;* §§$p<0.01$ *vs.* Cd.

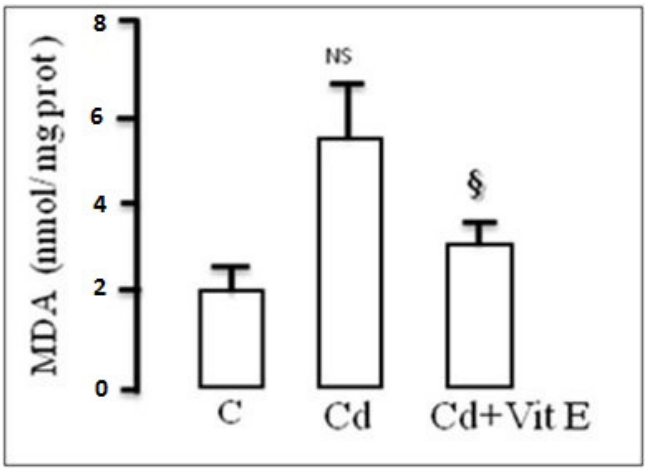

Figure 2: Effect of Cadmium and Vitamin E on lipid peroxidation in the liver.

Rats were daily administered with 10% ethanol (C), Cadmium (Cd), or Cadmium and Vitamin E (Cd/VitE) for 21days. Results are expressed by means ± S.E.M. (n=8); $^{\S}p<0.05$ vs. Cd.

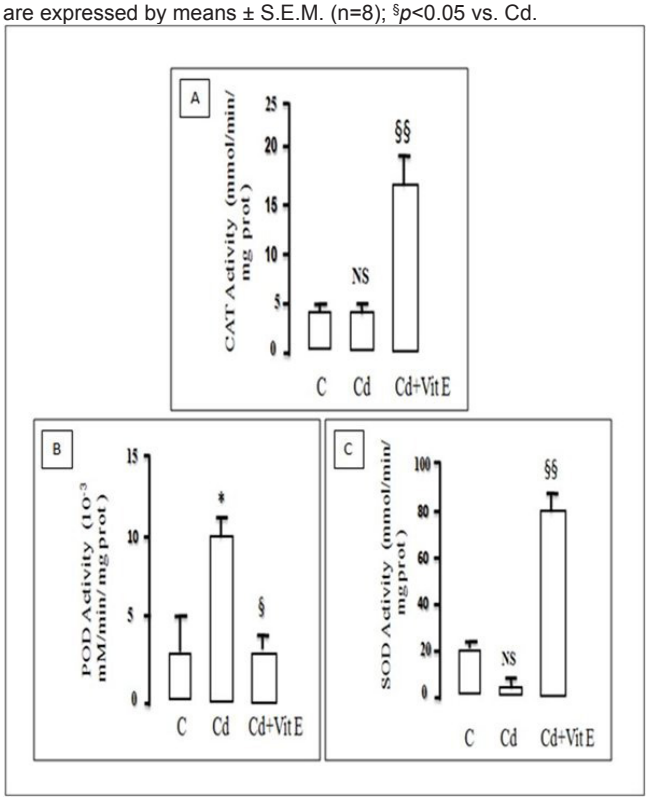

Figure 3: Effect of Cadmium and Vitamin E on liver antioxidant activities.

Rats were daily administered with 10% ethanol (C), Cadmium(Cd), or Cadmium and Vitamin E (Cd/VitE) for 21days. Catalase (Fig. 3A), peroxidase (Fig. 3B) and superoxide dismutase (Fig. 3C) activities were determined. Results are expressed by means ±S.E.M. (n=8);* $p<0.05$ vs. C; $^{\S\S}p<0.01$ vs. Cd, $^{\S}p<0.05$ vs. Cd.

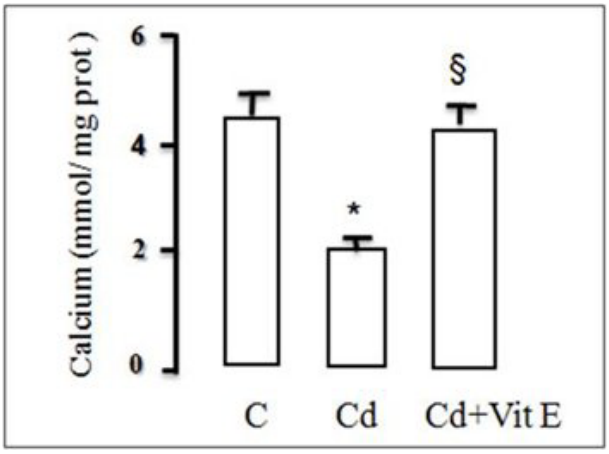

Figure 4: Effect of Cadmium and Vitamin E on calcium content.

Rats were daily administered with 10% ethanol (C), Cadmium (Cd), or Cadmium and Vitamin E (Cd/VitE) for 21 days. Results are expressed by means ±S.E.M. (n=8);*$p<0.05$ vs. C; $^{\S}p<0.05$ vs. Cd.

Effect of Cadmium and Vitamin E on calcium content

Treatment of rats with a high dose of Cd (15mg/kg *bw*), caused a significant decrease in intracellular liver calcium compared to the control group. Treatment with Vitamin E counteracted this decrease.

Effect of Cadmium and Vitamin E on liver Histology

Histopathological study exhibited that the typical hepatic architecture (Fig 5A) was altered by Cd as assessed by focal necrosis and inflammatory cell infiltration (Fig 5B). Co-treatment with vitamin E reversed the altered morphology as showed by normal hepatocytes with mild portal inflammation (Fig 5C).

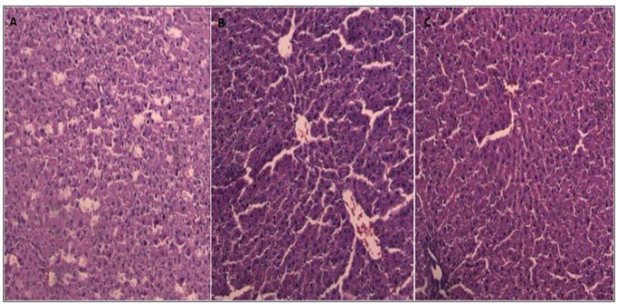

Figure 5: Effect of Cadmium and Vitamin E on liver Histology.

Liver section from control (10% ethanol) (Fig 5A), Cadmium (Fig. 5B)or Cadmium +vitamin E (Fig. 5C).(HEX 400).

DISCUSSION

In this study, our experiment showed clearly that Cd at a dose of 15mg/kg body weight administered in the diet induces an increase in the rate of MDA in the liver. They are consistent with those of [13] who claim that heavy metal increases the rate of liver TBARs. We also recorded a decrease of MDA in the liver after combined exposure of rats to Cd and vitamin E. Similar works [14, 15] confirm our observations, and since the Cd is considered as a metal that causes intracellular formation of ROS that attack cell membrane and organelles, Alpha-tocopherol may have enabled the formation of molecules that sequester Cd, like metallothionein, thereby preventing its distribution within hepatocytes and decreasing the rate of MDA.

Moreover, there was a significant decrease in intracellular calcium in the liver following administration of Cd alone. Indeed, it was demonstrated by [16] that Cd has a high affinity for calcium channels so it have a strong competition with $Ca^{2+}$for binding o the active site in the pore of the channel. This latter has an affinity for Cd^{2+} more than Ca^{2+} which reduces and / or inhibits the calcium influx either through the plasma membrane of the cell or through the endoplasmic membrane conducing to a lower intracellular Ca level in liver cells. We found that Vitamin E offered efficient protection and counteracted Ca decrease to near control. It is assumed that Vitamin E promoted the stimulation of the binding of Cd to metallothionein in these cells reducing the rate of free Cd and preventing its attachment at Calcium channels.

In a second step, our data showed that hepatic catalase activity remains stable in the group treated with Cd versus control group. Treatment with Vitamin E increased significantly the catalase activity in the liver. It has been suggested that Alpha-tocopherol, the active form of Vitamin E strengthened the Catalase activity in the liver of rats pretreated with high dose of Cd.Cd administration decreased SOD activity. This decrease can be explained by the installation of a severe oxidative stress induced by this heavy metal caused by the immobilization of Cd on its binding site in the metallothionein and /or because of its high content in cells which exceeded the binding capacity of metallothionein [17]. The decrease of SOD activity can be explained by the occupation of Cd in the binding site of transition metals such as zinc and copper. This reaction caused changes in its active site and transformed the SOD in an inactive form. Therefore, it is suggested that the stability of the rate of catalase in the liver is caused by an early location of this heavy metal in the active site of SOD which prevented its action on CAT.

Different studies have shown the direct relationship between Cd and oxidative stress resulting in decreased activity of SOD [18, 19, 20, 21, 22]. Co-treatment with vitamin E increased SOD activity when compared to the group treated with Cd alone. It is assumed in this case that Alpha-tocopherol could activate endogenous systems for the capture of Cd. These reactions reduced the attachment of Cd to SOD and increased its rate.

On the other hand, peroxidases which converted peroxides and hydroxyl radicalst o a non-toxic form (H_2O) detoxified ROS by reducing the level of peroxide. Peroxidases maintained also a stable supply of metabolic intermediates necessary for optimal function of antioxidant enzymes mainly the catalase. Indeed, we noticed an increase in total peroxidase in the liver compared to the control group after the administration of Cd alone. This is probably may be explained by an adaptive mechanism stimulating the peroxidase activity after chronic exposure to Cd [23, 24]. Vitamin E counteracted POD increase to near control. Our results confirm other research carried out the interesting role of Alpha-tocopherol as a protective substance against the dysfunction of endogenous antioxidant system of the liver [25, 26] . Moreover, histological data revealed that vitamin E protected the liver from Cd induced alteration of its regular architecture.

CONCLUSION

Our results underlines the fact that cadmium induces a disturbance in the normal rate of antioxidant enzymes, and the addition of vitamin E could reduce these adverse effects. Finally, α-tocopherol has a tendency to minimize this changes and the ability to improve cell survival and antioxidant defense capacity in the rat liver.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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ABREVIATIONS

BHT: 2,6,-di-tert-butyl-4-hydroxytoluene
Ca : calcium
CAT : catalase
Cd : Cadmium
DNPH : 2,4,dinitrophenyl hydrazine
H_2O_2 : hydrogen peroxide
HE: hematoxylin-eosin staining
MDA: malondialdehyde
PO: per orally
POD : peroxidase
ROS: Reactive oxygen species
IP : intraperitonial
SOD: superoxide dismutase
TBA: Thiobarbituric acid
TCA: trichloroacetic acid

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