

Protective Effect of Magnesium and Selenium on Cadmium Toxicity in the Isolated Perfused Rat Liver System

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Abstract- The isolated perfused rat liver (IPRL) model has been used into toxicology study of rat liver. This model provides an opportunity at evaluation of liver function in an isolated setting. Studies showed that Cd, in a dose-dependent manner, induced toxic effects in IPRL models, and these effects were associated with aminotransferase activity and lipid peroxidation. The aim of this study was to investigate whether Mg and/or Se could have protective effects against the Cd toxicity in the IPRL model. Male Wistar rats (9-10 weeks) weighing 260-300 gr were used in this study. They were randomly divided into 8 groups of 4-6 rats per cage. In group 1, liver was perfused by Krebs-Henseleit buffer without MgSO₄ (Control). Groups 2-8 were exposed to Mg, Se, Cd, Mg +Se, Cd + Mg, Cd + Se, Cd + Mg + Se respectively in Krebs-Henseleit buffer with no added MgSO₄. Biochemical changes in the liver were examined within 90 minutes, and the result showed that the exposure to Cd, lowered glutathione level, while it increased malondialdehyde level and aminotransferase activities in IPRL model. Mg administration during exposure to Cd reduces the toxicity of Cd in the liver isolated while Se administration during exposure to Cd did not decrease Cd hepatotoxicity. Nevertheless, simultaneous treatment with Se and Mg on Cd toxicity have strengthened protective effects than the supplementation of Se alone in the liver.

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Introduction

Cadmium (Cd) pollution was considered as a health risk in 1970, and World Health Organization (WHO) has identified renal dysfunction as the most important toxicity of Cd in 1992 (1). The International Agency for Research on Cancer (IARC) introduced Cd as a carcinogenic agent in humans (2). Primary exposure sources of Cd in the general population include tobacco smoking and food (3). Liver is the major target of Cd, so acute exposure to toxic doses of Cd results primarily liver injury (4). Many toxic effects of Cd seem to be due to the oxidative stress induced by following exposure to this metal (5). Since Cd is a

nontransition metal; therefore radical formation by Cd may be through some indirect mechanisms. Cd through mitochondrial dysfunction (6), depleting glutathione (GSH) (7) or by inhibiting antioxidant enzymes (8) causes enhances of free radicals (9) and lipid peroxidation (LPO) (10). Cd toxicity may also be due to depletion (11) or metabolic disorder of trace elements, such as Selenium (Se) (12). The Se is an essential trace element for antioxidant enzymes such as glutathione peroxidase and thioredoxin reductases (13). The glutathione peroxidase is an enzyme that is widely known to prevent polyunsaturated fatty acids against the oxidative damage generated by peroxides (14).

It is well established that Magnesium (Mg)

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treatment can have beneficial effects on oxidative stress (15,16) and LPO (17,18). Whereas Mg deficiency increases the levels of imposes oxidative damage in neuronal cells (19). It has been suggested that Mg by increasing levels of reduced GSH (20) and the activity of superoxide dismutase, (21) decreases free radicals and LPO generation. Moreover, Mg inhibits the activity of reduced NADPH oxidase and xanthine oxidase, the enzymes that produce superoxide radicals (22).

In the published literature, Se intake during Cd exposure demonstrated to have beneficial effects on Cd-induced toxicity (23-25). However, Limited experimental data point to beneficial effects of Mg against Cd toxicity (26). Thus, in the current study, we have attempted to investigate if the simultaneous administration of Se and Mg increases the protective effect of Se or Mg alone, on hepatotoxicity induced by Cd in the IPRL.

Materials and Methods

Chemicals

Cadmium chloride (CdCl_2) and Magnesium sulfate (MgSO_4) were provided from Merck (Darmstadt, Germany). Sodium selenite (Na_2SeO_3) was purchased from Sigma. Other materials used in the experiment were purchased from reliable companies.

Animals

Male rats (9 -10 weeks) weighing 260-300 grams were obtained from the vivarium section of the Department of Pharmacology, the Tehran University of Medical Sciences (Tehran, Iran). Room temperature was kept at 22°C and humidity maintained at 50%. Rats were allowed to become acclimatized to standard laboratory condition for at least 7 days, and standard food and water was provided. They were withdrawn Access of food 15 hr before the start of the experiment but were free access to water.

Experimental design

Animals were divided into 8 groups. Each group contained 4-6 male rats. In group 1, liver was perfused by Krebs-Henseleit buffer with no added MgSO_4 (Control). Groups 2-8 were exposed to Mg, Se, Cd, Mg-Se, Cd-Mg, Cd-Se, Cd-Mg-Se respectively in Krebs-Henseleit buffer Without MgSO_4 . After 10 minutes of washout (-30 ~ -20 min) and before adding Cd to the Krebs buffer, the

liver were as pre-treatment to exposed of Se and/or Mg (-20 ~ 0 min). After adding Cd to the buffer (0 min) perfusate samples were taken at regular 30-minute intervals.

Liver perfusion

The liver perfusion and all surgical procedures were performed according to Wolkoff *et al.*, (27). Briefly, the rats were anaesthetized by intraperitoneal injection of Ketamine 70mg/kg and Xylazine 15 mg/kg body weight. The anterior abdomen was cleaned with alcohol; the thorax was opened and both the hepatic portal vein (inlet) and the thoracic inferior vena cava (outlet) were cannulated. For successful perfusion, Heparin (500 units) can also be used to prevent blood clotting, although this is not obligatory. The liver output was perfused through a catheter cannulated into the portal vein. The perfusate was collected from a catheter placed in the superior vena cava via the right atrium. After cannulation, livers were perfused with a buffer containing (mM): 118.9NaCl, 4.76KCl, 1.19 KH_2PO_4 , 2.55 CaCl_2 , and 24.8 NaHCO_3 , at a pH of 7.25 to 7.4, without adding MgSO_4 . D-glucose (0.1% w/v) was added to provide energy source. The buffer was equilibrated with an O_2/CO_2 95:5 gas mixture and maintained at 37°C . The flow rate of perfusion was 2.5 ml/g liver/min.

Concentrations Cd, Se, Mg

To obtain the most suitable concentration of Se and Cd various concentrations used for Cd (10, 15, 20, 50,100 μM) and Se (2.5, 5, 25,50 μM). Finally, according to the changes in the activities of transaminase enzymes, concentrations 15 μM of Cd and 2.5 μM of Se were selected as the most suitable concentration. To study the effects of Mg, 0.29 g/l (1.2mM) magnesium sulfate was added to Krebs buffer without Mg.

Biochemical study

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the perfusion fluid samples were assayed by using a commercial kit from Teb Gostaran Hayan (Tehran, Iran). The LPO level in homogenate liver tissue was determined by the thiobarbituric acid method (28). GSH and total protein were estimated by the Kuo and Hook and the Bradford method respectively (29, 30).

Statistical analysis

Statistical analysis was performed by using the

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One-way Analysis of Variance (ANOVA) followed by post-hoc Tukey tests. All data were presented as mean \pm SE. Values were considered statistically significant when $P < 0.05$.

Results

Aminotransferase activity

When Se, Mg and Se Mg-Separately added to the solution, they did not induce any significant difference in the enzymes activity in comparison with the values of the control group ($P > 0.05$). Exposure to Cd caused a significant increase of AST and ALT activity compared to control ($P < 0.001$). When simultaneously Se and Cd added, it did not induce any significant difference in the transaminase enzymes activity in comparison with Cd-treated group ($P > 0.05$). When Mg supply added to Cd, the enzyme activity significantly

decreased (Figures 1 and 2). With simultaneous Se and Mg treatment, during exposure to Cd, a significant decrease in AST and ALT activity was observed in comparison to of the Cd treated group (Figures 1 and 2).

Malondialdehyde (MDA) concentrations

MDA levels were significantly increased ($P < 0.001$) in Cd exposed rats as compared to controls. Se alone had no effect on MDA increase induced by Cd ($P > 0.05$ compared to control), whereas Mg supply (Cd-Mg) alone reversed this change ($P < 0.001$). No significant difference in MDA concentration was found between Cd-Mg group and Cd-Mg-Se exposed rats. With simultaneous Se and Mg treatment, we noticed a complete prevention from the Cd-induced increase in MDA level (compared to control and Se group) (Figure 3).

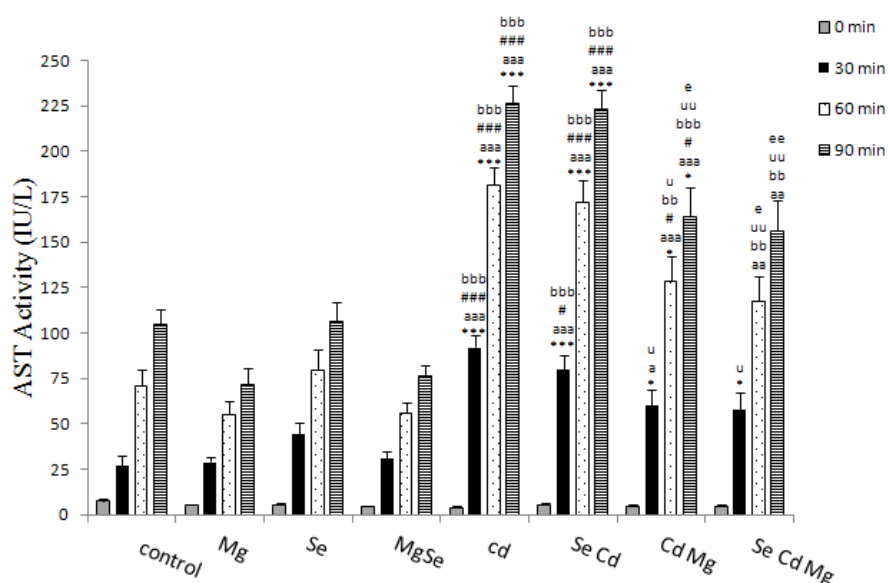


Figure 1. Effect of Cd with and without Se and/ or Mg on AST activities (minutes 0, 30, 60 and 90) in IPR model Data represent mean \pm SE (4–6 livers per group). Statistically significant differences from control: *** $P < 0.001$, * $P < 0.05$; from Mg: aaa $P < 0.001$, aa $P < 0.01$, a $P < 0.05$; from Se: ### $P < 0.001$, # $P < 0.05$; from Se-Mg: bbb $P < 0.001$, bb $P < 0.01$; from Cd: uu $P < 0.01$, u $P < 0.05$; from Cd-Se: ee $P < 0.01$, e $P < 0.05$

GSH concentrations

There was a significant decrease ($P < 0.001$) in GSH concentrations in liver tissue after Cd treatment in compared to the control group. The treatment of Cd-exposed liver with Se had no significant effect on the Cd-induced decrease in the GSH concentration (compared to the Cd group). GSH concentration in the Cd-Mg group was increased in compared to Cd group

($P < 0.01$), but it had a significant decrease in compare to control group ($P < 0.05$). Although co-treatment with Se and Mg had more protective effective than Mg alone in Cd-exposed livers but it was not significant. GSH concentration in Cd-Mg-Se group had no significant different with control and Se groups (Figure 4).

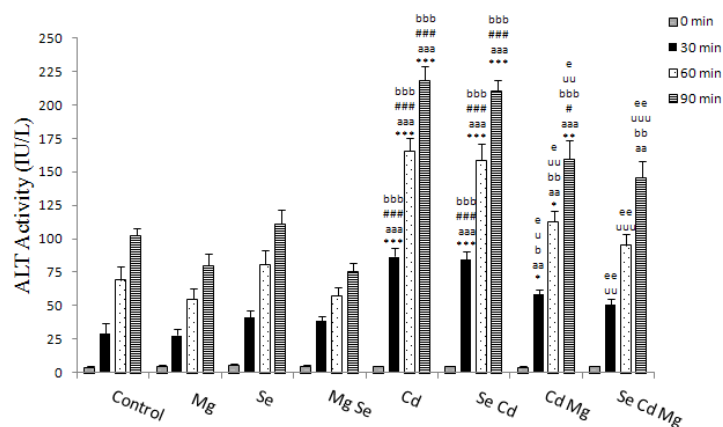


Figure 2. Effect of Cd with and without Se and/ Mg on ALT activities (minutes 0, 30, 60 and 90) in IPRL model. Data represent mean \pm SE (4–6 livers per group). Statistically significant differences from control: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; from Mg: aaa $P < 0.001$, aa $P < 0.01$; from Se: ### $P < 0.001$, # $P < 0.05$; from Se-Mg: bbb $P < 0.001$, bb $P < 0.01$, b $P < 0.05$; from Cd: uuu $P < 0.001$, uu $P < 0.01$, u $P < 0.05$; from Cd-Se: ee $P < 0.01$, e $P < 0.05$

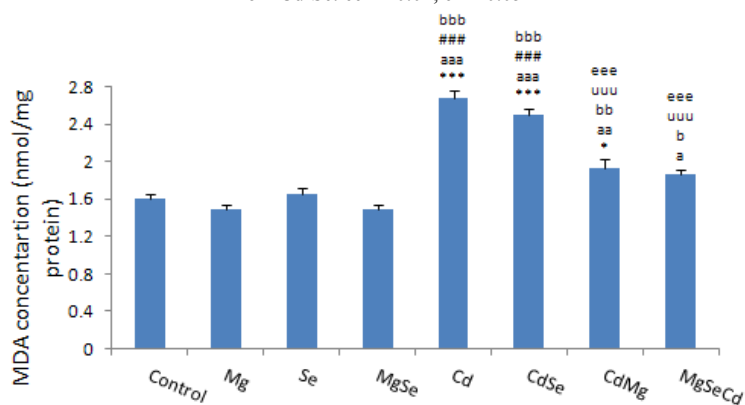


Figure 3. Effect of Cd with and without Se and / or Mg on MDA level in IPRL model. Data represent mean \pm SE (4–6 livers per group). Statistically significant differences from control: *** $P < 0.001$, * $P < 0.05$; from Mg: aaa $P < 0.001$, aa $P < 0.01$, a $P < 0.05$; from Se: ### $P < 0.001$; from Se-Mg: bbb $P < 0.001$, bb $P < 0.01$, b $P < 0.05$; from Cd: uuu $P < 0.001$; from Cd-Se: eee $P < 0.001$

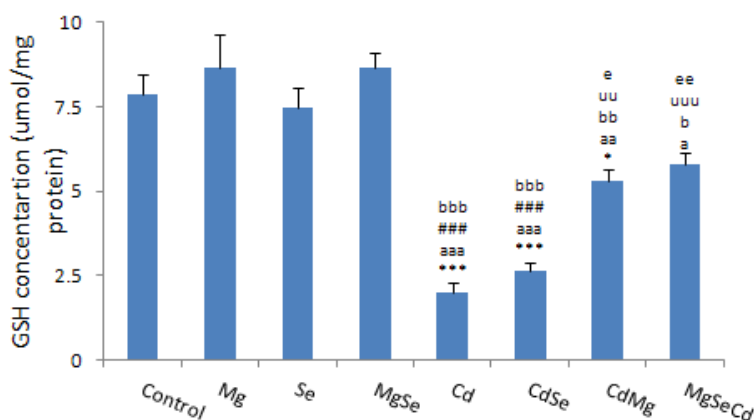


Figure 4. Effect of Cd with and without Se and / or Mg on GSH level in IPRL model. Data represent mean \pm SE (4–6 livers per group). Statistically significant differences from control: *** $P < 0.001$, * $P < 0.05$; from Mg: aaa $P < 0.001$, aa $P < 0.01$, a $P < 0.05$; from Se: ### $P < 0.001$; from Se-Mg: bbb $P < 0.001$, bb $P < 0.01$, b $P < 0.05$; from Cd: uuu $P < 0.001$, uu $P < 0.01$; from Cd-Se: ee $P < 0.01$, e $P < 0.05$

Discussion

In this study, in order to study the effects of Se and Mg on Cd-induced toxicity in IPRL model, we used ALT and AST biomarkers and MDA and GSH in order to evaluate the liver injury and for assessing the role of oxidative stress in Cd-induced hepatotoxicity, respectively.

In consistence with few previous studies (31,32), our results indicated a significant increase in activities of AST and ALT following having exposed Cd. It is suggested that the Cd increases free radicals (33) and LPO production (34,35) through damaging cell membranes (36), reducing antioxidant enzymes activities (37) and depleting glutathione (38). In our study, Cd caused liver damage through depleting GSH and increasing LPO. Se is a critical component for activities of antioxidant selenoenzymes (39). This element protects liver against Cd-induced toxicity by increasing antioxidant enzymes activities and decreasing MDA (40). In our study, using Se simultaneously with Cd improved GSH and MDA parameters slightly compare to the Cd group, but these changes were not statistically significant. It seems that the reason for non-effectiveness of Se on Cd toxicity largely depends on the Cd/Se ratio and the experimental protocol (41).

There have been a few studies on the effect of Mg on Cd-induced toxicity, as in previous studies Mg had reduced Cd-induced toxicity through reducing MDA level (42) or increasing GSH level (43). Mg deficiency in the *In vitro* (44) and *in vivo* (45) was followed by GSH decrease and increased LPO level. In fact, Mg as a cofactor of Gamma Glutamyl cysteine Synthetase and Glutathione Synthetase enzymes plays an important role in Glutathione biosynthesis (46).

According to our results, using Mg in Cd-Mg group reduced transaminase and MDA levels and also increased GSH level significantly comparing to the Cd group. Nevertheless, this improvement in the parameter's levels was significantly less than the control group. Likely, normal or increased physiological levels of extracellular Mg, through maintaining GSH levels as a substrate for Glutathione peroxidase, decreased LPO levels (47). The most important objective of this study was comparing separated and simultaneous effect of Se and Mg on Cd-induced hepatotoxicity in IPRL model. According to our study results, simultaneous use of Mg and Se during the expose time with Cd improves MDA and GSH level insignificantly compare to the Cd group (this improvement wasn't significant in compare to the

Cd-Mg group).

Most interestingly, although GSH and MDA level in Cd-Mg-Se group was lower than the control group and Se, but this disparity was not statistically significant. It has been reported that the simultaneous effect of Mg and Se is more powerful in antagonizing alcohol-induced oxidative stress in the liver than the effect of Mg alone (48). According to the previous findings, it can be said that Mg plays an important role in regulating Glutathione peroxidase dependent to the Se through modulating GSH and Se availability (49). Thus, on the basis of our results, we can conclude that Co-treatment of Mg and Se significantly enhances antioxidant defense and is more effective against Cd-induced hepatotoxicity, than the supplementation of Se alone. Moreover, treatment with Mg on Cd toxicity was more effective than the supplementation of Se alone. However, exact mechanisms of this cooperative effect need to be further investigated.

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