

EFFECTS OF ORAL AND INTRAPERITONEAL MAGNESIUM TREATMENT AGAINST CADMIUM-INDUCED OXIDATIVE STRESS IN PLASMA OF RATS

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Cadmium (Cd) has been recognised as one of the most important environmental and industrial pollutants, and up-to-date investigations have shown that one of the mechanisms of its toxicity is associated with the induction of oxidative stress. The aim of this study was to determine the connection between acute oral and intraperitoneal exposure to Cd and parameters indicative of oxidative stress in the plasma of rats, as well as to examine the potential protective effect of magnesium (Mg) in conditions of acute oral and intraperitoneal Cd poisoning.

The experiment was performed on male albino Wistar rats (n=40) randomly divided into control group, Cd_{or} group that received 30 mg kg⁻¹ b.w. Cd by oral gavage, Cd+Mg_{or} group that orally received 50 mg kg⁻¹ b.w. Mg one hour before oral Cd, Cd_{ip} group that received 1.5 mg kg⁻¹ b.w. Cd intraperitoneally, and Cd+Mg_{ip} group that intraperitoneally received 3 mg kg⁻¹ b.w. Mg 10 min before intraperitoneal Cd. The animals were sacrificed 24 h after treatment and the following parameters were measured: superoxide-dismutase activity, superoxide anion, total oxidative status, advanced oxidation protein products, and malondialdehyde.

All parameters of oxidative stress in rat plasma were negatively affected by Cd treatment with more pronounced negative effects after intraperitoneal treatment, with the exception of superoxide dismutase (SOD) activity. Although both oral and intraperitoneal Mg pretreatment had protective effects, more pronounced beneficial effects were observed after oral administration, since it managed to completely prevent Cd-induced changes in the investigated parameters. The observed results support the use of Mg as potential protective agent against toxic effects caused by Cd.

KEY WORDS: *bioelement supplementation, interactions between metals, mechanisms of toxicity*

Cadmium (Cd) has been recognised as one of the most important environmental and industrial pollutants of both natural and anthropogenic origin. The production and utilisation of Cd result in professional exposure to Cd, while food contamination and cigarette smoking contribute to exposure of the general population. It has also been estimated that exposure to this metal will increase in the decades to come (1). Cadmium has no uniform mechanism of toxicity (2-4).

It has been reported that one of the mechanisms is disturbance of prooxidant-antioxidant balance in tissues, which results in increased levels of reactive oxygen species (ROS) and oxidative damage of macromolecules as reviewed by Matović et al. (5). This can lead to various pathological conditions in humans and animals, such as hepatic and renal dysfunction, testicular damage, respiratory disorders, and cancer (6-9).

There are limited experimental data pointing out Mg ability to reduce organ Cd accumulation and its beneficial effects against Cd-altered bioelement levels and parameters of oxidative stress (10-12). The wide safety margin between beneficial and toxic concentrations of Mg in tissues and biological fluids suggests that Mg may be used to prevent and treat Cd poisoning. This potential is of great importance, having in mind that the therapy of Cd poisoning has not yet been established. However, the exact mechanisms of Mg beneficial effects against Cd poisoning are not completely understood.

The aim of this study was to determine how Mg affects parameters of oxidative stress in the blood of rats acutely poisoned with Cd. For this purpose we measured plasma enzyme superoxide dismutase (SOD) activity, superoxide anion ($O_2^{\cdot-}$) content, total oxidative status (TOS), malondialdehyde (MDA), and advanced oxidation protein products (AOPP) levels. Two routes of Cd exposure were chosen on the basis of two possible scenarios of human exposure to Cd: oral (or) as a model for exposure via food and water, and intraperitoneal (ip) as a model for parenteral exposure, i.e. inhalation. Our previous study (13) has shown that oral pretreatment with Mg had beneficial effects on all parameters of oxidative stress in the liver, suggesting gastrointestinal tract (GIT) as an important site of Cd and Mg interactions. However, the beneficial effect of Mg ip treatment observed in that study showed that competition between Cd and Mg in GIT can not be the only explanation for the protective effects of Mg against Cd poisoning. In this study we investigated the effects of oral and intraperitoneal Mg on parameters of oxidative stress rat plasma 24 h after Cd treatment in order to better understand possible protective mechanisms of Mg pretreatment against Cd toxicity.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of the Military Medical Academy for animal experiments. Procedures involving animals and their care were in compliance with Guidelines for Animal Studies no. 282-12/2002.

Animals

The study included 40 male Wistar rats weighing 170 g to 240 g, which were obtained from the Military

Medical Academy, Belgrade. The animals were housed in cages under standard laboratory conditions [temperature (22 ± 2) °C; relative humidity (50 ± 10) %] with light and dark cycles exchanging every 12 h. They had free access to water and received standard pelleted diet mixture (Veterinary Institute "Subotica", Subotica, Serbia). The diet contained 2.4 mg g⁻¹ Mg and 19.2 ng g⁻¹ Cd (as determined in our laboratory).

Experimental design

The animals were randomised into five groups of eight rats: control group, Cd_{or}, Cd+Mg_{or}, Cd_{ip}, and Cd+Mg_{ip} group. Oral and intraperitoneal doses of Cd producing toxic effects and Mg doses with possible protective effects were chosen on the basis of our previous experiments and literature data (10-15). For oral treatment, CdCl₂ and Mg(CH₃COO)₂ (Merck, Darmstadt, Germany) were dissolved in distilled water and the Cd_{or} group received a single Cd dose of 30 mg kg⁻¹ b.w. by orogastric tube, while the Cd+Mg_{or} group received a single Mg dose of 50 mg kg⁻¹ b.w. one hour before receiving oral Cd. A solution for intraperitoneal treatment was obtained in isotonic saline and the total injected volume in the peritoneal cavity was 0.5 mL. Rats in the Cd_{ip} group were intraperitoneally injected a single Cd dose of 1.5 mg kg⁻¹ b.w., while rats in the Cd+Mg_{ip} group received a single Mg dose of 3 mg kg⁻¹ b.w. 10 minutes before Cd injection. Control animals were unexposed to either Mg or Cd. The animals were killed under ether anaesthesia twenty-four hours after Cd poisoning and blood samples taken from the heart and collected into heparin tubes. One portion of collected blood was used for cadmium, magnesium, and zinc determination, while the other portion was centrifuged at 837xg for 15 min and various parameters of oxidative stress were determined in the obtained plasma.

Analytical procedures

All chemicals and reagents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Luis, USA). Parameters of oxidative stress in blood plasma SOD activity, $O_2^{\cdot-}$, MDA, AOPP, and TOS were measured using an ILAB 300+ analyser (Instrumentation Laboratory, Lexington, Massachusetts).

Plasma SOD (16) was assayed according to the Misra and Fridovich method (17) in which inhibition of epinephrine auto-oxidation by SOD in the examined sample is recorded as an increase in absorbance at 505 nm for 3 min. One unit of SOD activity is defined as

the activity that inhibits auto-oxidation of adrenalin by 50 %. The rate of nitroblue tetrazolium reduction (NBT) was monitored at 515 nm every 15 s within a minute to measure the level of $O_2^{\cdot-}$ (18). Lipid peroxidation (LPO) was evaluated by measuring MDA concentration according to a method described by Girroti et al. (19). This assay is based on the formation of a complex between thiobarbituric acid and MDA, which absorbs at 535 nm. AOPP levels, which indicate the level of ROS-mediated protein damage, were determined by the spectroscopic analysis of modified proteins at 340 nm (20) and expressed as chloramine-T equivalents ($\mu\text{mol L}^{-1}$). Total oxidative status was determined using the method described by Erel (21). The assay is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the formed ferric ion by xylenol orange.

For Cd, Mg, and Zn determination blood samples were mineralised with a mixture of HNO_3 and HClO_4 in a ratio 4:1, and Cd concentrations were measured by graphite furnace atomic absorption spectrophotometry (AAS, SpectrAA 220, GTA 110, Varian, Melbourne, Australia), while Mg and Zn concentrations were determined by flame atomic absorption spectrophotometry (AAS, apparatus GBC 932AA, Victoria, Australia). The accuracy and precision of analysis were established by the analysis of these elements in standard reference bovine liver from the National Bureau of Standards (NIST SRM 1577a bovine liver, National Institute of Standards and Technology, Gaithersburg, Maryland, USA).

Statistical analysis

For statistical analysis we used SPSS (version 11.5 for Windows) and MedCalc[®] 12.1.4. The one-sample Kolmogorov-Smirnov test showed that only Cd and Mg concentrations were normally distributed and for these parameters the results were presented as means \pm SD and statistical evaluation of the data was performed using a one-way analysis of variance (ANOVA). Other parameters were presented as medians and ranges and statistical evaluation was performed using Kruskal-Wallis nonparametric test followed by post-hoc Conover test for pairwise comparison. Furthermore, Spearman's correlation analysis was used to determine the relationship between SOD activity and Zn levels in blood. Statistical significance was defined as $P < 0.05$.

RESULTS

SOD activity

Cadmium given both orally or intraperitoneally decreased SOD activity significantly compared to control values, while no significant difference was observed in SOD activity between the Cd_{or} and Cd_{ip} groups. Oral Mg co-treatment managed to prevent any significant changes in SOD activity since there were no significant difference between the $\text{Cd}+\text{Mg}_{\text{or}}$ group and control group, whereas *i.p.* Mg treatment resulted in higher SOD activity compared with the Cd_{ip} group although SOD activity was still significantly lower than in the control group (Table 1).

$O_2^{\cdot-}$ and TOS levels

Regardless of the route of administration, Cd induced a significant increase in both $O_2^{\cdot-}$ and TOS levels compared to controls, and this increase was much higher in the group that received Cd intraperitoneally for both parameters. Oral Mg managed to completely prevent changes in $O_2^{\cdot-}$ and TOS levels, keeping them within control range. Intraperitoneal Mg co-treatment induced a significant decrease in $O_2^{\cdot-}$ levels compared to the Cd_{ip} group, but could not maintain $O_2^{\cdot-}$ levels within control range. On the other hand, intraperitoneal Mg managed to prevent the increase in TOS levels, as control and $\text{Cd}+\text{Mg}_{\text{ip}}$ TOS levels did not differ significantly (Table 1).

MDA and AOPP levels

Both oral and intraperitoneal Cd treatment resulted in a significant increase in MDA and AOPP levels compared to controls, and these levels were significantly higher in the group treated intraperitoneally when compared to oral treatment. Oral administration of Mg in Cd-poisoned rats significantly decreased the level of both parameters to values observed in control group. Intraperitoneal administration showed the same positive effect on AOPP levels as oral, while MDA levels in the $\text{Cd}+\text{Mg}_{\text{ip}}$ group were significantly lower than in the Cd_{ip} group, but higher than in controls (Table 1).

Cd, Mg, and Zn concentrations

Table 2 shows that Cd blood levels in both intraperitoneally treated groups were significantly higher than in the control and orally treated groups. Oral Cd treatment did significantly affect blood Cd

Table 1 The effect of Cd or Cd+Mg treatment on the superoxide dismutase (SOD) activity, superoxide anion (O_2^-) content, total oxidative status (TOS), malondialdehyde (MDA) and advanced oxidation protein products (AOPP) levels in plasma

Parameter		Control	Cd _{or}	Cd+Mg _{or}	Cd _{ip}	Cd+Mg _{ip}
SOD / IU	Median	137 ^a	130 ^b	138 ^a	123 ^b	134 ^c
	Range	131 to 139	127 to 134	135 to 139	119 to 133	127 to 137
O ₂ ⁻ / $\mu\text{mol min}^{-1} \text{L}^{-1}$ red. NBT	Median	87.5 ^a	107 ^b	78 ^a	284 ^c	162 ^b
	Range	60 to 99	101 to 128	62 to 96	234 to 370	110 to 190
TOS / $\mu\text{mol L}^{-1}$	Median	28.3 ^a	49 ^b	26.6 ^a	68.3 ^c	24 ^a
	Range	15.6 to 39.8	41.6 to 63.2	21.4 to 44.4	46 to 83.8	22.6 to 34.4
MDA / $\mu\text{mol L}^{-1}$	Median	0.68 ^a	1.14 ^b	0.68 ^a	1.82 ^c	1.14 ^b
	Range	0.46 to 0.93	0.93 to 1.69	0.51 to 0.93	1.36 to 1.93	1.06 to 1.44
AOPP / $\mu\text{mol L}^{-1}$ of chloramine-T equivalents	Median	42.83 ^a	43.78 ^b	43.53 ^a	45.47 ^b	43.27 ^a
	Range	41.06 to 43.22	43.28 to 54.94	42.28 to 44.39	43.11 to 47.39	42.22 to 44.56

Control group - non-treated animals, Cd_{or} group - rats intoxicated orally with 30 mg kg⁻¹ b.w. Cd; Cd+Mg_{or} group - rats given orally 30 mg kg⁻¹ b.w. Cd and 50 mg kg⁻¹ b.w. Mg one hour prior to Cd treatment; Cd_{ip} group - rats intraperitoneally injected a single dose of 1.5 mg kg⁻¹ b.w. Cd; Cd+Mg_{ip} group - rats intraperitoneally injected a single dose of 3 mg kg⁻¹ b.w. Mg 10 minutes prior to i.p. treatment with Cd. The values are expressed as medians and ranges and statistical evaluation was performed using Kruskal-Wallis nonparametric test followed by post-hoc Conover test for pairwise comparison. Means not sharing the same letter are significantly different ($P < 0.05$).

levels, and neither did Cd+Mg co-treatment. Blood Mg was in the control range in all groups. Table 2 also shows changes in blood Zn of control and treated rats. A significant decrease was observed in both the Cd_{or} and Cd_{ip} groups compared to control, with no significant difference between these two particular groups. However, oral Mg administration managed to prevent Cd-induced changes in Zn levels.

DISCUSSION

All investigated parameters of oxidative stress in rat plasma were negatively affected by Cd treatment. These observed changes are in accordance with our previous results and results of other authors who have suggested that Cd toxicity is mediated by decrease in

antioxidant enzymes, production of reactive oxygen species and lipid peroxidation (13, 14, 22, 23). However, more pronounced negative effects of Cd, except for SOD activity, were observed in rats that were treated intraperitoneally. This can be explained by significantly higher blood Cd levels in Cd_{ip} than in Cd_{or} group.

Our study shows that Cd influences plasma SOD activity in rats, but that this effect does not depend on the route of exposure. Similarly, no route-dependent changes in SOD activity were found in the liver of Cd-poisoned rats (13). The antagonism between Cd and Zn has been investigated for many decades, and it is known that Cd can replace Zn in many vital enzymatic reactions (24-26). This suggests that Cd may induce changes in SOD activity by interacting with Zn, which is present in the active centre of SOD isoenzymes beside Cu and Mg. Blood Zn levels in

Table 2 The effect of Cd or Cd+Mg treatment on the Cd, Mg and Zn levels in plasma

Parameter		Control	Cd _{or}	Cd+Mg _{or}	Cd _{ip}	Cd+Mg _{ip}
Cd / $\mu\text{g L}^{-1}$		11.38±3.57 ^a	25.37±4.50 ^a	31.86±9.02 ^a	77.42±35.61 ^b	123.38±26.24 ^b
Mg / mg L ⁻¹		33.53±3.29 ^a	33.70±2.59 ^a	35.72±3.34 ^a	35.78±2.62 ^a	34.33±2.29 ^a
Zn / mg L ⁻¹	Median	5.65 ^a	4.95 ^b	5.65 ^a	5.23 ^b	5.04 ^b
	Range	5.42 to 7.6	4.03 to 6.15	5.17 to 6.51	4.95 to 5.68	4.22 to 5.5

Control group - non-treated animals, Cd_{or} group - rats intoxicated orally with 30 mg kg⁻¹ b.w. Cd; Cd+Mg_{or} group - rats given orally 30 mg kg⁻¹ b.w. Cd and 50 mg kg⁻¹ b.w. Mg one hour prior to Cd treatment; Cd_{ip} group - rats intraperitoneally injected a single dose of 1.5 mg kg⁻¹ b.w. Cd; Cd+Mg_{ip} group - rats intraperitoneally injected a single dose of 3 mg kg⁻¹ b.w. Mg 10 minutes prior to i.p. treatment with Cd. The values for Cd and Mg are expressed as mean values \pm S.D. while values for Zn are expressed as medians and ranges. Means not sharing the same letter are significantly different ($P < 0.05$).

both the Cd_{or} and Cd_{ip} treated rats were significantly lower than control, but no significant difference was found between routes of exposure, which may explain similar levels of SOD activity in these groups. This suggests that mainly affects SOD through interaction with Zn. This finding is supported by Spearman's correlation analysis that revealed a positive correlation between blood Zn and SOD activity ($\rho=0.339$, $P=0.035$).

This study was focused on how Mg affects blood parameters of oxidative stress in rats acutely poisoned with Cd. The results show that oral Mg pre-treatment was effective in reducing this stress: SOD activity, O₂⁻, TOS, MDA, and AOPP were all restored to control values. These results are in accordance with our previous investigations that demonstrated beneficial effects of Mg on parameters of oxidative stress in the liver (13, 27). This protective effect of oral Mg supplementation could be explained by Mg and Cd interactions in the gastrointestinal tract (GIT) where Mg competes with Cd for divalent metal transporters. Recent studies (28, 29) have shown that Mg absorption from the GIT happens through divalent cation channels TRPM 7 and TRPM 6 (transient receptor potential melastation-related 7 and 6), which have been demonstrated to be primarily Mg²⁺ channels, but are also implicated in Cd trafficking. Besides, Mg transporter protein-MagT2 can also be engaged in other divalent cation transport. Recent data (29) also suggested that ancient conserved domain protein 2 is a non-selective divalent cation transporter which favours transfer of divalent cations transport in case of Mg deficiency. Therefore, excessive Mg intake can significantly decrease Cd transport through these channels. However, this principle alone can not explain our results, as there was no significant difference in blood Cd levels between the Cd_{or} and Cd+Mg_{or} groups. Concentration of Cd in these treated groups were even similar to those obtained in control group, but it should be pointed out that Cd was determined only 24 h after the treatment when both Cd and Mg were already distributed in the organism. The positive effects of Mg on SOD activity can be explained by the protective effect of Mg on Zn levels in blood since oral Mg administration counteracts Cd-induced Zn reduction in blood. One explanation for this phenomenon may be that Mg prevents Cd-induced extensive loss of Zn via urine, which was observed in our previous study on rabbits (30).

Intraperitoneal Mg treatment manifested beneficial effects on O₂⁻ and TOS levels and subsequently on

MDA and AOPP levels, while it did not exert any effect on SOD activity. The most pronounced changes were observed in TOS and AOPP levels: Mg_{ip} administration managed to completely remove Cd toxic effects on these parameters, restoring them to control values. Since Mg_{ip} treatment showed a beneficial effect on TOS [that includes reactive oxygen metabolites such as hydrogen peroxide (H₂O₂) and lipid hydroperoxides (LOOH)], but not on SOD activity, it can be hypothesised that Mg affects other antioxidant enzymes such as glutathione peroxidase (GPX). This enzyme plays an important role in lipid hydroperoxide reduction to their corresponding alcohols and in the decomposition of hydrogen peroxide to water. Previous studies have shown that Cd adversely affects GPX activity in different tissues such as serum, liver, and kidney (31-33), which can result in elevated TOS levels, as observed in this study. On the other hand, Vernet et al. (34) found that Mg deficiency decreased GPX activity, suggesting a positive role of supplemental Mg. The beneficial effect of Mg_{ip} treatment on O₂⁻ levels might also be explained by enhanced GPX activity and subsequent decrease in H₂O₂ levels. Having in mind that H₂O₂ is also a product of superoxide anion radical dismutation, its decrease can lead to lowered levels of O₂⁻ as a substrate. Intraperitoneal Mg pretreatment totally reversed Cd-induced changes in AOPP levels and managed to counteract, at least partly, Cd-induced changes in MDA levels. Under the same experimental conditions, no effect on SOD activity was observed, perhaps as a consequence of unchanged blood Zn levels after intraperitoneal Mg supplementation.

Our findings show that both oral and intraperitoneal Mg pretreatment have a protective effect against Cd poisoning, but also indicate that the route of Mg administration affects interactions between Cd and Mg, as oral administration provides more beneficial effects. These differences can be explained by the fact that after oral treatment, Mg is present in the GIT where it can modify Cd absorption. Furthermore, oral Mg pretreatment in this study prevented blood Zn drop while intraperitoneal treatment did not.

CONCLUSION

The results of this study show that acute intraperitoneal administration of Cd has much more potent toxic effect than oral Cd administration on parameters of oxidative stress in rat plasma. Our

results also show that oral and intraperitoneal Mg pretreatment can have a beneficial effect on Cd-induced oxidative stress in rat plasma, but this effect was more pronounced after oral administration. As no specific therapy has been adopted for Cd poisoning, the observed beneficial effects of both oral and intraperitoneal Mg encourage further use of Mg as protection against the toxic effects caused by Cd.

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Sažetak

UTJECAJ ORALNOG I INTRAPERITONEALNOG TRETMANA MAGNEZIJEJEM NA OKSIDATIVNI STRES U PLAZMI ŠTAKORA TROVANIH KADMIJEM

Kadmij (Cd) jedan je od najvažnijih industrijskih onečišćivača i onečišćivača životne sredine. Dosadašnja su istraživanja pokazala da je jedan od mehanizama toksičnosti ovog metala nastanak oksidativnog stresa. Cilj ovog rada bio je ispitati utjecaj akutnog oralnog i intraperitonealnog trovanja kadmijem na parametre oksidativnog stresa u plazmi štakora i eventualni zaštitni efekt magnezija (Mg) u danim uvjetima.

Eksperiment je izveden na 40 mužjaka albino Wistar štakora podijeljenih u ove skupine: kontrolnu, Cd_{or} skupinu koja je primila 30 mg kg⁻¹ t.m. Cd oralnom gavažom, Cd+Mg_{or} skupinu koja je oralno tretirana s 50 mg kg⁻¹ t.m. Mg jedan sat prije oralno apliciranog Cd, Cd_{ip} skupinu koja je primila 1,5 mg kg⁻¹ t.m. Cd intraperitonealno i Cd+Mg_{ip} skupinu koja je intraperitonealno tretirana s 3 mg kg⁻¹ t.m. Mg 10 min prije intraperitonealno apliciranog Cd. Životinje su žrtvovane 24 h nakon tretmana i izmjereni su ovi parametri: aktivnost enzima superoksid dismutaze, koncentracije superoksidnog aniona, uznapredovalih produkata oksidacije proteina i malondialdehida.

Kadmij je negativno djelovao na sve ispitivane parametre oksidativnog stresa u plazmi štakora pri čemu su negativni efekti bili izraženiji nakon intraperitonealne primjene, s izuzetkom efekta na aktivnost enzima superoksid dismutaze (SOD). Iako je Mg pokazao pozitivan učinak i nakon oralne i nakon intraperitonealne primjene, izraženiji pozitivni efekti uočeni su nakon oralnog tretmana magnezijem. Rezultati ovog rada upućuju na mogućnost primjene Mg u prevenciji toksičnih efekata Cd.

KLJUČNE RIJEČI: interakcije između metala, mehanizmi toksičnosti, suplementacija bioelementima

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