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Bull Vet Inst Pulawy 59, 541-545, 2015
DOI:10.1515/bvip-2015-0081

Magnesium supplements affect selected cadmium toxic actions and uptake of repeated doses of cadmium

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Received: July 6, 2015 *Accepted: November 17, 2015*

Abstract

The importance of magnesium supplements on organ retention of cadmium and allometric parameters after repeated exposure to cadmium chloride were studied in male Wistar rats. Magnesium chloride was given *via* drinking water (500 mg Mg/L) to rats exposed intragastrically to cadmium chloride (labelled with cadmium 109) at a daily dose corresponding to 25 mg/kg diet for 7, 14, 21, and 28 d. Supplements of magnesium temporarily decreased cadmium retention in the duodenum and liver. No significant differences in cadmium retention were evidenced in the kidneys and testicles. The supplements of magnesium also retain more of the body weight gains and restore the relative liver and testicle weight in rats intoxicated with cadmium. Comparison of the present results with earlier reports suggests a relationship between doses of magnesium and cadmium; higher doses of cadmium need more magnesium to overcome toxic action of the heavy metal.

Keywords: rat, cadmium, magnesium, interaction, organs.

Introduction

Cadmium is non-essential and toxic in humans, animals, plants, and microorganisms. In the environment, cadmium is persistent and cannot be broken down into less toxic substances. In spite of decreasing cadmium emissions, recent reports have shown that cadmium deposition into soil still exceeds its removal, which could result in increased future exposure through food. Cadmium absorbed by humans and animals mainly accumulates in the kidneys and skeleton where it may serve as a source of exposure later in life. The biological half-life of cadmium is long (decades) and its concentrations in most tissues increase with age (10, 16, 21).

The risk of cadmium contamination depends on its environmental dietary concentrations and the magnitude and duration of exposure. The uptake of cadmium is affected by the type of diet and nutritional status. Food or feed is believed to be the main source of non-occupational exposure to cadmium and its uptake depends on dietary habits; diets rich in fibre and shellfish increase cadmium content in the body. The long biological half-life of cadmium means that even a small decrease in its bioavailability may result in reducing distant toxicological manifestations (10, 16, 21).

Research on the effects of nutrients on the bioavailability of cadmium has produced growing evidence for the beneficial role of selenium, calcium, zinc, and iron, and dietary ingredients in reducing cadmium uptake and toxicity (2, 4, 8, 11, 13, 16, 17, 18, 22, 23).

The studies arguing the importance of magnesium and cadmium interaction are not well established. Van Barneveld and Van den Hamer (20) found that feeding a magnesium-deprived or magnesium-supplemented diet did not affect cadmium and lead metabolism in mice but more recent studies have provided evidence for the role of magnesium in cadmium body burden and toxic action. These findings showed that magnesium supplementation

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of rabbits, mice, and rats exposed to subacute doses of cadmium reduced cadmium retention and toxicity in several organs of the animals (4, 5, 8, 9, 14, 15, 22, 23).

In previous studies (9) we exposed Wistar rats to 10 ppm of cadmium along with 500 mg Mg/L in drinking water, which resulted in significant changes in cadmium distribution in the body. On the basis of those studies, we examine here whether coadministered magnesium would influence the body distribution of higher doses of cadmium (25 ppm) and ameliorate toxic effects of this metal in rats. Cadmium and magnesium were given for various periods of time to assess effects of the Mg exposure time in a supplemented diet on Cd retention in the body including influences on selected histopathological and allometric parameters. The experiments involved cadmium chloride labelled with ^{109}Cd to follow cadmium body distribution over time.

Material and Methods

Male Wistar rats initially weighing from 205 g to 230 g were used, and the total number of subjects was 75. The animals were randomly assigned into three dietary groups (the control ─group I, Cd treated – group II, and Mg plus Cd treated– group III) each of 25 rats after an acclimatisation period of one week. The animals were housed in polycarbonate cages and fed on a standard rodent chow LSM *ad libitum* (Fodder Manufacture Motycz, Poland). Chemical analysis showed that this diet contained about 1.780 g Mg/kg and 0.04 µg Cd/kg. Free access to food and tap water was allowed throughout the experiment. Rats in groups I and II were given tap water comprising about 19 mg Mg/L whereas rats in group III received the same water supplemented with dissolved magnesium chloride (POCH, Poland) up to 500 mg Mg/L. The total daily dose of magnesium given to each rat corresponded to about 70 mg/rat/day. The animals were on these diets for the whole experimental period and the consumption of feed and tap water was recorded daily for the duration of the study. Stock solutions of cadmium chloride (Acros Organics, Belgium) were made up in deionised water. $^{109}CdCl_2$ (The Institute of Atomic Energy, POLATOM, Poland) was added to prepare dosing solutions of known specific activity. Each rat in groups II and III was administered cadmium chloride intragastrically in a 0.5 mL water solution comprising about 20 kBq per rat daily for 7, 14, 21, and 28 d except weekends. The daily dose of cadmium corresponded to 25 mg Cd/kg diet. Body weight gains and organ to body ratios were recorded weekly during the 28 d feeding period.

Animals were euthanised by immersion in gaseous carbon dioxide 7, 14, 21, and 28 d post-dosing. Blood was withdrawn by cardiac puncture to plastic vials coated with EDTA to prevent clotting. The organs for testing were removed, trimmed free of fat, dried with a filter paper, weighed and homogenised for radiocadmium estimation.

Histological analysis included portions of the liver, kidney, and testicle which were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin.

Radiocadmium activity was measured in blood, duodenum, liver (using a piece of the largest lobe), kidney, spleen, heart, one testicle, brain, and thigh muscles by a Wizard 1480 automatic scintillation counter (Perkin Elmer, USA).

The data obtained in the duodenum without contents, liver, kidney, and testicle were expressed as a percentage of the total of radiocadmium administered. The results of the three groups examined were compared using ANOVA. When ANOVA indicated significant differences among groups, then pairwise comparisons were made by Tukey's method. Differences were considered significant if $P < 0.05$.

Results

The results are averages for five animals euthanised at each interval. Exposure of rats to cadmium (group II) and cadmium and magnesium (group III) did not markedly affect feed intake as compared to that in non-treated rats. However, rats supplemented with magnesium consumed visibly more water in comparison to those in groups I and II (data not shown). Table 1 shows that rats treated with cadmium gained significantly less weight after two-, three-, and four-week exposures as compared to non-treated rats whereas significantly lower body weight gains in the Mg plus Cd group in comparison to the non-treated group was found only after a three-week exposure.

Table 2 shows the ratios of organ weight to body weight in all rats examined. The data indicate a statistically significant increase in the relative liver weight after one–week exposure to cadmium as compared to that in the Mg-treated and non-treated rats. In the case of testicles, rats exposed only to cadmium revealed significant decreases in relative testicle weights after one-, two-, three-, and four-week exposures as compared to those found in the non-treated and Mg-plus-Cd-exposed animals. No statistically significant changes were found between non-treated rats and those exposed to cadmium plus magnesium. The relative renal and splenic weight (data are not shown) was similar in all examined groups.

Table 3 shows the deposition of cadmium 109 in the duodenum, kidneys, liver, and testicles collected at 7, 14, 21, and 28 d of treatment with Cd or Mg plus Cd. The concentrations of cadmium in the blood, muscles, heart, and brain were below 0.001% of the total dose administered at each stage of the experiment, so they were not included in the table.

Table 1. Total body weights (g) and weight gains (%)

Exposure time	Non-treated	Cd	Mg plus Cd
0	235 ± 10[a]	239 ± 16[a]	234 ± 20[a]
1 week	287 ± 17 (22.4%)[a]	256 ± 10 (7.5%)[a]	279 ± 27 (18.9%)[a]
2 weeks	343 ± 14 (46.3%)[a]	281 ± 14 (17.8%)[b]	305 ± 25 (30.7%)[ab]
3 weeks	425 ± 27 (80.9%)[a]	303 ± 22 (26.9%)[b]	327 ± 39 (40.1%)[b]
4 weeks	451 ± 37 (92.2%)[a]	331 ± 21 (38.7%)[b]	374 ± 40 (60.0%)[ab]

The data are expressed as the mean ± SD, N = 5
Cd – rats exposed to cadmium; Mg plus Cd – rats exposed to magnesium and cadmium; [a-b] – means that do not share a common superscript letter in lines are significantly different ($P < 0.05$)

Table 2. Organ weight to body weight ratio (%)

	Liver				Testicles			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
Non-treated	3.98 ± 0.34[a]	3.88 ± 0.32[a]	3.92 ± 0.25[a]	3.81 ± 0.41[a]	0.92 ± 0.13[a]	0.91 ± 0.15[a]	0.89 ± 0.08[a]	0.90 ± 0.13[a]
Cd	4.98 ± 0.35[b]	4.33 ± 0.22[a]	4.15 ± 0.09[a]	4.04 ± 0.26[a]	0.62 ± 0.07[b]	0.74 ± 0.11[b]	0.69 ± 0.09[b]	0.64 ± 0.08[b]
Mg plus Cd	4.41 ± 0.24[ab]	3.97 ± 0.19[a]	3.66 ± 0.20[a]	3.79 ± 0.37[a]	0.96 ± 0.10[a]	0.96 ± 0.10[a]	0.93 ± 0.13[a]	0.84 ± 0.10[a]

Explanation as in Table 1 except that means which do not share a common superscript letter in columns are significantly different at $P < 0.05$

Table 3. Retention of cadmium 109 (percentage of total dose) in rat tissues expressed as percentage of total dose in the duodenum (1g), kidneys, liver, and testicles (whole organs)

		Duodenum	Kidneys	Liver	Testicles
One week	Cd	0.098 ± 0.018[a]	0.040 ± 0.007[a]	0.291 ± 0.072[a]	0.002 ± 0.001[a]
	Mg plus Cd	0.088 ± 0.022[a]	0.041 ± 0.004[a]	0.320 ± 0.078[a]	0.002 ± 0.001[a]
Two weeks	Cd	0.065 ± 0.005[a]	0.042 ± 0.006[a]	0.293 ± 0.094[a]	0.003 ± 0.001[a]
	Mg plus Cd	0.045 ± 0.007[b]	0.047 ± 0014[a]	0.271 ± 0.087[a]	0.002 ± 0.001[a]
Three weeks	Cd	0.082 ± 0.008[a]	0.038 ± 0.007[a]	0.277 ± 0.052[a]	0.003 ± 0.001[a]
	Mg plus Cd	0.053 ± 0.011[b]	0.048 ± 0.008[a]	0.301 ± 0.075[a]	0.003 ± 0.001[a]
Four weeks	Cd	0.045 ± 0.006[a]	0.046 ± 0.009[a]	0.336 ± 0.045[a]	0.003 ± 0.001
	Mg plus Cd	0.023 ± 0.005[b]	0.044 ± 0.007[a]	0.234 ± 0.039[b]	trace levels

Means which do not share a common superscript at each time interval are significantly different at $P < 0.05$,
trace levels – measurements of cadmium 109 close to the limit of detection

The highest retention of cadmium 109 expressed as the percentage of the total dose was found in the liver and kidneys. The duodenal and testicle cadmium deposition was markedly lower and in the case of the duodenum decreased with time in the two groups examined; however, decreases were more pronounced in Mg-than in Cd-exposed rats and led to significant differences after two-, three-, and four-week exposures. By contrast, the renal cadmium content in Cd- and Mg-plus-Cd-treated rats remained unaltered over time. Similarly, testicular cadmium content was unchanged within a four-week exposure except for a drop to trace levels in Mg-plus-Cd-exposed rats. Hepatic cadmium content was similar for a three-week exposure in the two groups tested but decreased significantly thereafter in Mg-plus-Cd-treated rats as compared to that in the Cd group.

Discussion

In the present study, the rats fed a magnesium-supplemented diet consumed daily about 70 mg Mg/kg b.w. Gaal *et al*. (7) reported that high dietary magnesium

supplements have a beneficial effect including improved digestibility of feed better reproduction, and a shortened service period in cows and sows. Moreover, there are no experimental data on detrimental physiological changes caused by long-term magnesium administration over standard levels (18).

Based on the data shown in Tables 1 and 2 it was found that rats treated with cadmium demonstrated lower body weight gains and relative hepatic and testicle weights in comparison to the non-treated rats. Supplements of magnesium restored the relative weight of testicles to that reported for the controls. In addition, magnesium supplements diminished the body weight losses resulting from cadmium administration. The beneficial action of magnesium on the relative weight of testicles may be in accordance with the results of Djukic-Cosic *et al.* (5) who found that magnesium stimulates production of glutathione (GSH) *de novo*, which indicates a protective role of magnesium against the toxic action of cadmium and a magnesium-mitigated decrease in lipid peroxidation produced by cadmium (2).

The harmful effect of cadmium on testicle relative weight was not accompanied by any blood or histopathological alterations. Our findings may support the view that, in toxicology studies, analysis of relative organ weights and body weight gains may be an important endpoint for identification of potentially harmful effects of chemicals even though morphological changes are absent (1).

The ratio of kidney weight to body weight (not shown) was not affected by the exposure to cadmium. This finding may seem a little surprising because the kidneys are considered one of the primary organs to be affected adversely following chronic cadmium exposure (15). A likely explanation for this controversy may result from the metabolism of cadmium within the body, especially when the absorption from the gastrointestinal tract is considered. Zalups and Ahmad (22) reported that cadmium is taken up by the liver *via* the hepatic portal vein, stored in this organ, and then transported as a metallothionein complex directly to the kidneys where it accumulates with time, and renally-toxic action of cadmium may be visible after a longer period.

The retention of cadmium in the duodenum and liver of the rats supplemented with magnesium fell after a four-week co-exposure. This finding agrees in part with the data showing that magnesium pre-treatment decreases cadmium content in several organs of rabbits and mice administered with subacute doses of this metal (10 mg/kg b.w.). It was also reported that magnesium decreased renal cadmium content after intoxication with an acute single dose of cadmium (20 mg/kg) 4 h and 6 h post-dosing (3, 4, 8). However, these data contrast with the presented results showing no significant changes in renal cadmium deposition after magnesium supplementation. The disagreement between these findings may be caused by the difference between cadmium doses. The interaction between a single dose of magnesium and cadmium in the kidneys was shown to be more affective shortly after dosing than after a prolonged exposure to relatively high doses of cadmium (5).

Significant decreases in intestinal cadmium deposition indicate that supplements of magnesium may reduce the uptake of cadmium from the gastrointestinal tract and thus diminish the body burden and toxicity of cadmium. A lowered cadmium deposition in the liver of supplemented rats unmanifest until four weeks of exposure may suggest that, under the conditions of this experiment, magnesium supplements effectively influence cadmium uptake after a prolonged co-exposure of the two metals.

The mechanism of magnesium and cadmium interaction at the gastrointestinal level is not fully understood because of limited experimental data. Matovic *et al.* (13, 14) suggest the magnesium modifies cadmium uptake by affecting the intracellular leak of cadmium ions from intestinal lumen to portal blood, reducing the presence of cadmium in the blood.

To summarise, the results presented support previous findings which showed that magnesium supplements may play a beneficial role in animals exposed to cadmium. Moreover, the results suggest that the effectiveness of magnesium supplements depends on cadmium dosage and duration of exposure. Comparison of the present results with those reported earlier (8) may indicate that when the dosage of cadmium increases, the advantageous influence of magnesium seems to be reduced. Thus, it suggests a relationship between the doses of magnesium and cadmium, and suggests that a relative increase in magnesium dosage may be more effective in lowering the body burden and toxic action of cadmium.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Animal Rights Statement: The authors declare that the experiments on animals were conducted in accordance with local Ethical Committee laws and regulations as regard care and use of laboratory animals. The studies were approved also by the Second Ethics Committee for Animal Experimentation in Lublin, Poland. Resolution No. 16/2014.

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