HET

Human and Experimental Toxicology
000(00) 1–8

DOI: 10.1177/0960327110362703
het.sagepub.com
SAGE

Cadmium induced-oxidative stress in pituitary gland is reversed by removing the contamination source

Eliana A Miler, Silvana I Nudler, Fernanda A Quinteros, Jimena P Cabilla, Sonia A Ronchetti and Beatriz H Duvilanski

Abstract

Cadmium (Cd^{2+}) is one of the most important environmental contaminants and acts as an endocrine disruptor. Previously, we have demonstrated that the simultaneous administration of Cd^{2+} and melatonin (Mel) in drinking water impaired metal-induced oxidative stress in rat anterior pituitary gland. The aim of this study was to investigate if a treatment started after the toxic manifestations of Cd^{2+} became evident could reverse the effects of the metal. Animals exposed to Cd^{2+} (5 parts per million [ppm], 30 days) were treated with Mel or without the metal during the next 1 or 2 months. Cd^{2+} exposure increased the expression of heme oxygenase-1 (HO-1), a biomarker of oxidative stress, and an a posteriori Mel treatment reversed oxidative stress induced by Cd^{2+}. This effect was also observed 1 month after metal removal. The Cd^{2+}-induced increase in metallothionein-1 (MT-1) and nitric oxide synthase 1 (NOS1) expression were also reversed by metal removal. In addition, serum prolactin and luteinizing hormone levels affected by Cd^{2+} exposure were normalized. Considering that the manifestations of Cd^{2+} intoxication become evident only after a certain period of metal accumulation, these results show that metal removal is enough to reverse Cd^{2+} effects in anterior pituitary gland and bring to light the relevance of moving away the individual from the contamination source.

Keywords

cadmium, oxidative stress, anterior pituitary gland

Introduction

Cadmium (Cd^{2+}) is one of the most important environmental contaminants. Levels of this heavy metal are increasing in the environment due to industrial and agricultural practices.[1,2] Besides occupational exposure, the largest potential sources of Cd^{2+} intoxication for humans are food, drinking water, and cigarette smoke.[3]

In humans, Cd^{2+} has a long biological half-life (15–30 years)[4] mainly due to its low rate of excretion from the body. Consequently, the metal accumulates over time in blood, kidney, and liver,[4-7] as well as in reproductive organs.[7-11]

Human exposure to this metal is associated with increased incidences of several cancer types and other diseases.[12] Liver and kidney have been considered the major target organs for Cd^{2+} toxicity. These tissues have been used as standards for delimiting metal toxic concentrations.[2] However, several recent reports indicate that, both in human and animal models, chronic exposure to low doses of the metal can cause neurobehavioral disturbances even in the absence of hepatic or renal damage.[13,14] In addition, we have demonstrated that chronic administration of low doses

Departamento de Química Biológica, IQUIFIB, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

Corresponding author:
Beatriz H Duvilanski, Departamento de Química Biológica – IQUIFIB, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Buenos Aires (C1113AAD), Argentina.
Email: neuroend@ffyb.uba.ar

of Cd^{2+} induced oxidative stress in rat anterior pituitary gland but not in liver.[15]

It is well known that Cd^{2+} can act as an endocrine disruptor strongly affecting the reproductive organs.[16] Exposure of rodents to this metal resulted in a down-regulation of pituitary hormones including gonadotropins, prolactin (PRL), adrenocorticotropin hormone, growth hormone, and thyroid-stimulating hormone.[17] Moreover, various Cd^{2+} effects on reproductive system have been described, but definitive conclusions about its actions on target tissues vary depending on the experimental model and the dosage employed.

Among the mechanisms of action of the metal, it has been demonstrated that Cd^{2+} stimulates free radical production, resulting in oxidative deterioration of lipids, proteins, and DNA, and prompting various pathological conditions in human and other animals.[18] In rat anterior pituitary cells in culture, Cd^{2+} inhibits PRL secretion and induces apoptosis and both effects are abolished by antioxidants.[19] Cd^{2+}-induced oxidative stress has been evaluated by different biomarkers like lipid peroxidation and mRNA expression of heme oxygenase-1 (HO-1).[15] Metallothioneins (MTs) were also determined.[20,21] HO-1 is an important stress biomarker[22] involved in bilirubin and biliverdin synthesis, which in turn constitute two important antioxidant agents. MTs are involved in the detoxification of certain heavy metals and are free radical scavengers too.[23-25] MT-1 and MT-2 isoforms are expressed in most tissues and are induced by heavy metals, oxidant agents, hormones, inflammation, and stress. MT-3 was originally identified in the brain.[26] However, this isoform was also detected in peripheral organs outside the brain, like the dorsolateral lobe of the prostate, testis, tongue, and kidney.[27,28] MT-4 is mainly found in skin.[29]

Cd^{2+} can affect nitric oxide (NO) production, either increasing or decreasing it. There are experimental data supporting two different alternative hypotheses: NO protects against Cd^{2+} and that NO mediates Cd^{2+} toxicity.[30,31] In anterior pituitary Cd^{2+} also stimulates nitric oxide synthase 1 (NOS1) expression in vivo and in vitro.[15,30] Moreover, MTs, metals, and NO show a close interrelationship. MTs can scavenge NO and in this way reduce the sensitivity of cells to toxic levels of NO. In turn, NO acts as mediator and regulator of MTs induction.[32,33]

We have previously demonstrated that the administration of Cd^{2+} (5 parts per million [ppm]) in drinking water during 30 days induced oxidative stress in rat anterior pituitary gland (produced lipid peroxidation and increased HO-1 and NOS1 mRNA expression), confirming in vitro results. The in vivo effects were impeded when an antioxidant such as melatonin (Mel) was simultaneously administered.[15] Mel has been successfully used to reduce oxidative stress in many systems[34] due to its ability to scavenge hydroxyl, carbonate, and various organic radicals as well as peroxynitrite and other reactive nitrogen species.[35-37]

To date, most of the studies aimed at ameliorating the toxic effects of metals apply different treatments before or simultaneously to metal intoxication.[38,39] However, in Cd^{2+}-exposed populations, the clinical manifestations of metal intoxication become evident only after a certain period of its accumulation.

The aim of this study was to investigate if the toxic effects of Cd^{2+} can be reversed by a treatment started after the chronic exposure to the metal. We studied whether an a posteriori treatment with Mel or merely the end of metal exposure were able to reverse the oxidative stress induced in vivo by Cd^{2+} in anterior pituitary gland.

We demonstrated that Cd^{2+}-induced oxidative stress in anterior pituitary gland can be reversed both by metal removal from drinking water and by an a posteriori Mel treatment.

Methods

Materials

$CdCl_2$ was purchased from Mallinckrodt Chemical Works (St. Louis, MO, USA). Melatonin was obtained from Sigma (St. Louis, MO, USA). GoTaq DNA polymerase, random hexamers, and deoxynucleotide triphosphates (dNTP) were provided by Promega (Madison, WI, USA). TRIzol and molecular biology reagents were purchased from Invitrogen (Carlsbad, CA, USA).

Animals and treatments

Adult male Wistar rats (180–200 g) were used. Animals were kept with controlled conditions of light (12:12 hours light/dark cycle) and temperature (21–24°C). Food and water were supplied ad libitum. All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Rats were divided into six groups of six to eight animals per group, receiving Cd^{2+} (5 ppm), Mel (0.4 μg/mL) through drinking water or only water (H_2O) as described in Table 1. For comparison purposes, we chose the same Cd^{2+}dose used in a previous work.[15]

Table 1. Experimental design for cadmium (Cd^{2+}) exposure and melatonin (Mel) treatment[a]

	Days 1–30	Days 30–60	Days 60–90
Control	H_2O	H_2O	
Cd^{2+}	H_2O	Cd^{2+}	
Mel	H_2O	Mel	
Cd^{2+}/Mel	Cd^{2+}	Mel	
Cd^{2+}/H_2O	Cd^{2+}	H_2O	
$Cd^{2+}/H_2O/H_2O$	Cd^{2+}	H_2O	H_2O

[a] Rats were exposed or not (control) to $CdCl_2$ 5 ppm in drinking water (Cd^{2+}). After 1 month of Cd^{2+} exposure, 1 group was treated with Mel (0.4 μg/mL) in drinking water (Cd^{2+}/Mel) for 1 month. In the other groups of rats exposed to Cd^{2+}, the metal was removed from the drinking water and animals were sacrificed 1 (Cd^{2+}/H_2O) or 2 months ($Cd^{2+}/H_2O/H_2O$) later.

Table 2. Pimers used for polymerase chain reaction

Gene	Primer		Product size (bp)
HO-1	Forward	5' TGCTCGCATGAACACTCTG3'	123
HO-1	Reverse	5' TCCTCTGTCAGCAGTGCC3'	
MT-1	Forward	5' GAATTCCGTTGCTCCAGATTCACCAGATC 3'	327
MT-1	Reverse	5' GAATTCTCACATGCTCGGTAGAAAACGG 3'	
NOS-1	Forward	5' ATCGGCGTCCGTGACTACTG 3'	92
NOS-1	Reverse	5' TCCTCATGTCCAAATCCATCTTCTTG 3'	
GAPDH	Forward	5' TGCACCACCAACTGCTTA 3'	176
GAPDH	Reverse	5' GGATGCAGGGATGATGTTC 3'	

Stock solution of Mel was prepared in ethanol; final ethanol concentration in drinking water was 0.0015%. Therefore, all groups received 0.0015% ethanol in drinking water. Water consumption was measured every 2 days and animal weight was registered once a week. After the end of each treatment, animals were killed by decapitation between 11 am and 12 noon and anterior pituitary gland and liver were carefully and quickly removed. Trunk blood was collected for PRL and luteinizing hormone (LH) assays.

Reverse transcription (RT) and semi-quantitative PCR

RNA isolation. Tissues were removed from decapitated animals and immediately homogenized with TRIzol reagent. After isolation, total RNA from tissues was spectrophotometrically quantified at 260 nm. RNA integrity was checked in formaldehyde/formamide gel electrophoresis.

RT and PCR reactions. First strand cDNA was synthesized with Moloney murine leukemia virus (M-MLV) reverse transcriptase in RT buffer containing 5.5 mM $MgCl_2$, 0.5 mM dNTP, 2.5 μM random hexamers, and 3.125 U/μL M-MLV reverse transcriptase. Reactions were done in a final volume of 12 μL containing 5 μg RNA. The reverse transcription reaction was run at 37°C for 50 min and reverse transcriptase was inactivated by heating the samples at 70°C for 15 min before the PCR reactions. To check for genomic contamination, the same procedure was performed on samples in a reaction solution lacking reverse transcriptase.

Specific primers for HO-1, MT-1, NOS1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed from published sequences[15,40] with Oligo Perfect designer software (Invitrogen) and are detailed in Table 2. GAPDH was used as an endogenous control. Then, samples were thermocycled for PCR amplification (Mastercycler, Eppendorf, Hamburg, Germany). The reaction mixture contained GoTaq PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.625 U GoTaq polymerase, and 300 nM of each primer. RT-PCR methods were utilized to determine relative changes in mRNA expression. Reactions were subjected to a varying number ($n = 16$–40) of cycles of PCR amplification (melting phase 94°C for 30 sec, annealing 55°C for 60 sec, and extension 72°C for 45 sec for HO-1, NOS1, and GAPDH, and melting phase 94°C for 60 sec, annealing 60°C for 30 sec and extension 72°C for 30 sec for MT-1) to find out the optimum cycle number within the linear range for PCR amplification. In all cases, each PCR reaction was hot-started 2 min at 94°C and finished with an

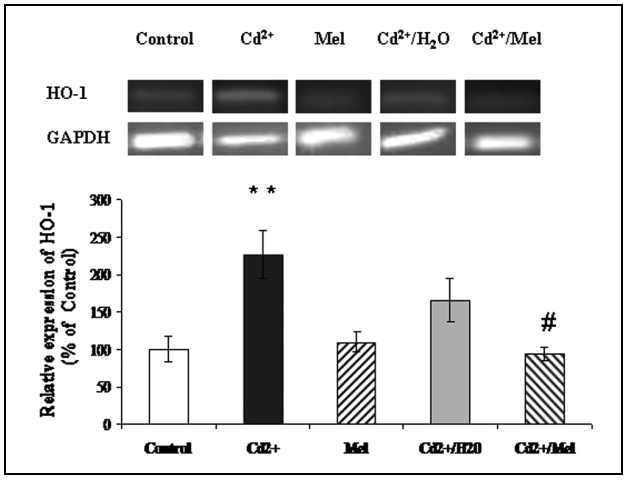

Figure 1. Melatonin (Mel) treatment reversed cadmium (Cd^{2+}) effects on hemeoxygenase-1 (HO-1) mRNA expression. Rats were exposed or not (control) to $CdCl_2$ 5 ppm in drinking water (Cd^{2+}). After 1 month, one group of rats exposed to Cd^{2+} received Mel (0.4 μg/mL) in drinking water (Cd^{2+}/Mel) while the other group received water without the metal (Cd^{2+}/H_2O) during the next month. Rats of Mel group (Mel) received Mel for 1 month. HO-1 mRNA levels were determined by semi-quantitative PCR. Top, a representative semi-quantitative PCR. Bottom, average densitometric values. Bars represent mean ± S.E.M. of HO-1 densitometric values normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; $n = 4$–8); ANOVA followed by Tukey-Kramer's test, $^{**}p < .01$ vs. control, $\#p < .05$ vs. Cd^{2+}.

elongation phase of 10 min at 72°C. The optimum cycle number for GAPDH resulted to be 26 for liver and 30 for anterior pituitary gland. HO-1, MT-1, and NOS1 mRNA amplicons from both tissues were obtained after 40 PCR cycles. Amplified products collected at various cycles were analyzed by electrophoresis in 2% agarose-ethidium bromide gels.

Analysis of semi-quantitative PCR and immunoblot data

The intensity of PCR products signals was determined by digital image analysis using the Gel Pro Analyzer (Media Cybernetics, LP, Silver Spring, MD, USA) software for Windows. To allow statistical comparison of results from different experiments, HO-1, MT-1, and NOS1 levels were normalized to the value of the GAPDH amplified band in each lane.

Hormone determination

PRL and LH were measured by a double-antibody radioimmunoassay[41] using reagents provided by National Hormone and Pituitary Program. The intra- and inter-assay coefficients of variation were lower than 10%.

Statistical analysis

Results are expressed as mean ± SEM and evaluated by one-way ANOVA followed by Tukey-Kramer's or Student-Newman-Keuls' tests. Differences between groups were considered significant if $p < .05$.

Results were confirmed by at least three independent experiments.

Results

No significant differences in water consumption and animals' weight were observed between groups (data not shown).

HO-1, MT-1, and NOS1 mRNA expression

HO-1 is an inducible enzyme that is strongly upregulated by oxidative stress. To address whether an a posteriori Mel treatment or the cessation of Cd^{2+} exposure were effective to reverse Cd^{2+}-induced oxidative stress, the effects of the metal on HO-1 mRNA expression was studied. Rats exposed to Cd^{2+} in drinking water for 1 month were (a) treated with Mel (Cd^{2+}/Mel) or (b) without the metal (Cd^{2+}/H_2O) for another month. Cd^{2+} exposure for 1 month significantly increased HO-1 mRNA expression in anterior pituitary gland (Figure 1). Mel treatment did not affect HO-1 expression and its administration after Cd^{2+} exposure (Cd^{2+}/Mel) was effective in reducing HO-1 mRNA expression to control levels (Figure 1). Rats exposed to Cd^{2+} for 1 month and subsequently for another month without the metal (Cd^{2+}/H_2O), which were used as control of Cd^{2+}/Mel group in these experiments, showed a partial reversion of Cd^{2+}-increased HO-1 expression (Figure 1). Considering this last result, we examined if a total reversion of Cd^{2+} effects on HO-1 expression could be achieved after a longer period of time without the metal. The expression of this enzyme was determined 2 months after Cd^{2+} exposure ($Cd^{2+}/H_2O/H_2O$). This period of time was enough to completely reverse Cd^{2+}-elicited effects on HO-1 mRNA expression (Figure 2).

Taking into account the results of HO-1 mRNA expression showed above, we then study if the cessation of metal exposure can per se reverse Cd^{2+} effects on other oxidative stress parameters. We studied Cd^{2+} effects on MTs, which scavenge free radicals and metals. Cd^{2+} exposure significantly enhanced MT-1

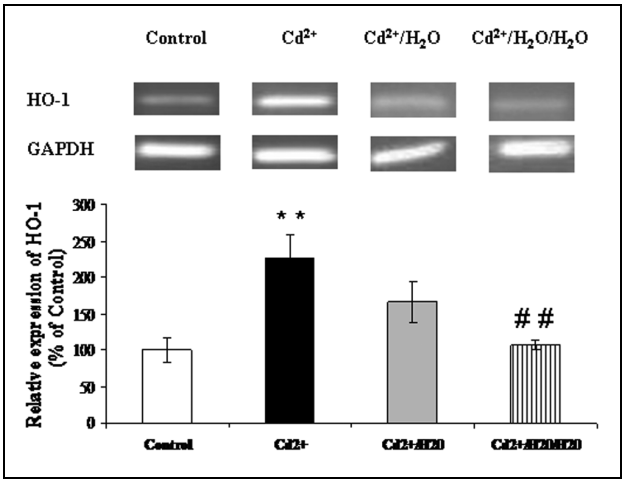

Figure 2. Cadmium (Cd^{2+}) removal from drinking water reversed the effects of the metal on hemeoxygenase-1 (HO-1) mRNA expression. Rats were exposed or not (control) to $CdCl_2$ 5 ppm in drinking water (Cd^{2+}). After 1 month of Cd^{2+} exposure, the metal was removed from drinking water and rats were sacrificed one (Cd^{2+}/H_2O) or 2 months ($Cd^{2+}/H_2O/H_2O$) later. HO-1 mRNA levels were determined by semi-quantitative PCR. Top, a representative semi-quantitative PCR. Bottom, average densitometric values. Bars represent mean ± S.E.M. of HO-1 densitometric values normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; $n = 4$–8); ANOVA followed by Tukey-Kramer's test, $^{**}p < .01$ vs control, $\#\#p < .01$ vs Cd^{2+}.

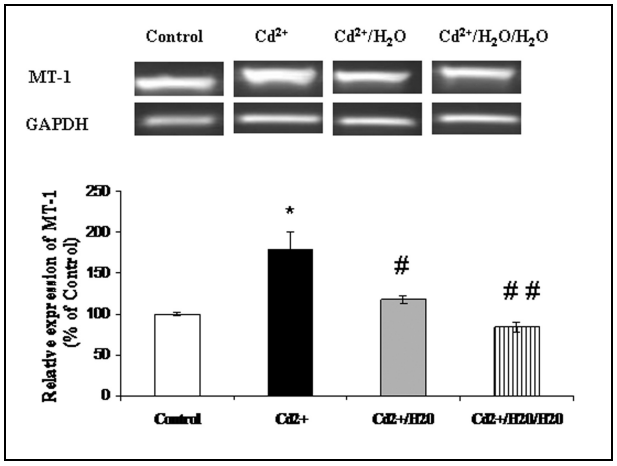

Figure 3. Cadmium (Cd^{2+}) removal from drinking water reversed the effects of the metal on metallothionein-1 (MT-1) mRNA expression. Rats were exposed or not (control) to $CdCl_2$ 5 ppm in drinking water (Cd^{2+}). After 1 month of Cd^{2+} exposure, the metal was removed from drinking water and rats were sacrificed one (Cd^{2+}/H_2O) or 2 months ($Cd^{2+}/H_2O/H_2O$) later. MT-1 mRNA levels were determined by semi-quantitative PCR. Top, a representative semi-quantitative PCR. Bottom, average densitometric values. Bars represent mean ± S.E.M. of MT-1 densitometric values normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; $n = 4$-8); ANOVA followed by Tukey-Kramer's test, $^{*}p < .05$ vs control, $\#p < .05$, $\#\#p < .01$ vs Cd^{2+}.

mRNA expression in anterior pituitary gland (Figure 3) and 1 month after metal exposure was enough to reverse the effect of Cd^{2+} (Cd^{2+}/H_2O). Similar results were obtained when the cessation of metal exposure was for a period of 2 months ($Cd^{2+}/H_2O/H_2O$).

Cd^{2+} administration significantly increased NOS1 mRNA expression in anterior pituitary gland.[15] One month after the end of Cd^{2+} exposure (Cd^{2+}/H_2O), NOS1 mRNA expression levels partially decreased and a complete reversion was obtained after 2 months ($Cd^{2+}/H_2O/H_2O$; Figure 4).

Cd^2 toxicity has been extensively studied in liver.[2,20] Previously, we have showed that Cd^{2+} administration (5 ppm for 30 days) did not affect HO-1 and NOS1 mRNA expression in liver.[15] Accordingly, here we also observed that MT-1 mRNA expression was not affected in liver after Cd^{2+} exposure (data not shown).

PRL and LH levels

PRL and LH serum levels decreased after in vivo Cd^{2+} exposure (Table 3) confirming previous in vitro results.[19] The effects of the metal on hormone secretion were completely reversed 1 month after the end of Cd^{2+} exposure (Cd^{2+}/H_2O).

Discussion

Since the manifestations of metal intoxication usually become evident after a certain period of accumulation, it is important to know if an a posteriori treatment can reverse or lessen the toxic effects produced by metals. Considering this, we studied the effect of an a posteriori treatment with Mel or the effect of metal removal on Cd^{2+}-induced oxidative stress in anterior pituitary gland. For comparison purposes, we chose a Cd^{2+} dose used in a previous report.[15] This concentration, apart from being the lowest dose that induced changes in pituitary hormones levels,[17] also mimics the most frequent type of human exposure (a low and chronic intoxication). Mel concentration was selected based on the dose that human use for their treatments (3 mg/day). We showed that Cd^{2+}-induced oxidative stress was sensitive to an a posteriori Mel treatment. This hormone was able to reverse Cd^{2+} effects on HO-1 expression

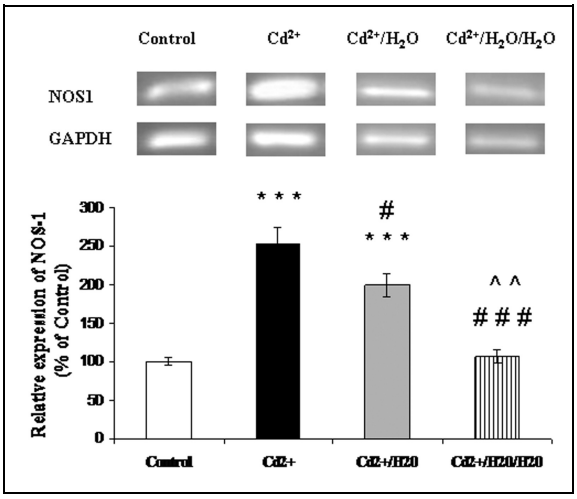

Figure 4. Cadmium (Cd^{2+}) removal from drinking water reversed the effects of the metal on nitric oxide synthase 1 (NOS1) mRNA expression. Rats were exposed or not (control) to $CdCl_2$ 5 ppm in drinking water (Cd^{2+}). After 1 month of Cd^{2+} exposure the metal was removed from drinking water and rats were sacrificed 1 (Cd^{2+}/H_2O) or 2 months ($Cd^{2+}/H_2O/H_2O$) later. NOS1 mRNA level were determined by semi-quantitative PCR. Top, a representative semi-quantitative PCR. Bottom, average densitometric values. Bars represent mean ± S.E.M. of NOS1 densitometric values normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; $n = 4$–8); ANOVA followed by Tukey-Kramer's test. *$p < .001$ vs control, #$p < .05$, $p < .001$ vs Cd^{2+}, ΛΛ$p < .01$ vs Cd^{2+}/H_2O.

in anterior pituitary gland even if it was administrated after metal exposure. This effect of Mel was similar to that observed after simultaneous treatment,[15] confirming its antioxidant properties. In addition, it is important to remark that the cessation of metal exposure also reversed Cd^{2+} effects on the expression of this oxidative stress biomarker. However, more time was needed to completely normalize this parameter.

Several studies support a protective role of MTs against Cd2+-elicited cell toxicity.[42] Lee et al. demonstrated that Cd2+ exposure increased the levels of MT-1 mRNA expression in various tissues such as dorsal prostate, kidney, and testis. In addition, Cd2+ exposure increases NOS1 mRNA expression in anterior pituitary gland[15] and stimulates NO production in anterior pituitary cells.[30] In the present work, Cd2+ increased MT-1 and NOS1 gene expression in this tissue. Our results confirm that the eradication of the contamination source could be enough to reverse Cd2+-induced oxidative stress since both parameters were reversed after a particular time without metal exposure.

Table 3. Cadmium (Cd^{2+}) removal from drinking water reversed the effects of the metal on plasma prolactin (PRL) and luteinizing hormone (LH) levels[a]

	PRL (ng/mL)	LH (ng/mL)
Control	18.7 ± 5.0	62.4 ± 10.6
Cd^{2+}	6.3 ± 0.5[b]	15.3 ± 6.3[b]
Cd^{2+}/H_2O	23.5 ± 2.8[c]	64.3 ± 13.3[c]

[a] Rats were exposed or not (control) to $CdCl_2$ 5 ppm in drinking water (Cd^{2+}). After 1 month of Cd^{2+} exposure, the metal was removed from the drinking water and rats were sacrificed 1 (Cd^{2+}/H_2O) month later. PRL and LH plasma levels were measured by radioimmunoassay and their concentration was expressed in ng/mL; ANOVA followed by Student-Newman-Keuls' (PRL) or Tukey-Kramer's (LH) tests.
[b] $p < .05$ vs control.
[c] $p < .05$ vs Cd^{2+}.

Liver and kidney, traditionally considered the main targets of Cd^{2+} toxicity, have been used to study risk levels of this metal in the population.[2,43] At the Cd^{2+} dose used in this work, none of the parameters studied were affected in liver, suggesting that the endocrine system may be more sensitive to Cd^{2+}. Similar results have been observed in other tissues.[13,14]

Regarding the effects of the metal on hormone release, the cessation of Cd^{2+} exposure for 1 month was effective to completely reverse the decrease in PRL and LH serum levels induced by the metal. Similar results were observed in vitro.[19] The present results suggest a direct effect of Cd^{2+} on hormone release since metal removal per se is enough to restore the functionality of the gland.

These results led us to question whether the mere elimination of the toxic factor could be enough to reverse the harmful effects of Cd^{2+}. Though different half-lives have been reported, it is well known that the Cd^{2+} persistence time in kidney and liver is very long (15–30 years) mainly due to its low rate of excretion.[2,44] However, Cd^{2+} clearance rate in different tissues seems to be variable[45] and although Cd^{2+} accumulates in anterior pituitary gland,[46,47] no report investigates its clearance rate from this tissue. Therefore, it may be possible that the reversion of Cd^{2+} effects in anterior pituitary gland after metal removal could be a consequence of a high rate of clearance. In addition, the rate of cell renovation could be another factor determining the diminution of oxidative stress induced by Cd^{2+}. It has been well studied that cell renovation rate in anterior pituitary gland is of about 1 month[48] while in liver is of 300–500 days.[49] However, the reversion of Cd^{2+}-induced oxidative damage could not only be explained by cell renovation since at

hypothalamic level, where cells are mainly from nervous origin with a long half-life, these oxidative stress biomarkers were stimulated by Cd^{2+} (5 ppm for 30 days) and a reversion was also observed (preliminary results). Future studies are necessary to determine the clearance rate from these tissues.

In conclusion, in the present study, we demonstrated for the first time that both, an a posteriori Mel treatment and the cessation of metal exposure can reverse Cd^{2+} oxidative effects in anterior pituitary gland. Although the last condition requires some more time to reverse the oxidative stress parameters evaluated, only 1 month is enough to reverse Cd^{2+} effects on PRL and LH release, restoring the physiology of the gland. Our results highlight the relevance of moving away the individual from the contamination source as one of the first steps for reversing the toxic effect of the metal, at least concerning anterior pituitary gland physiology. Our perspectives are focus in the study of the possible reversion of Cd^{2+} effects after longer periods of exposure.

Authors' note

The first two authors contributed equally to this work.

Acknowledgments

We are grateful to Dr Graciela Diaz-Torga (IByME, CONICET) and Dr Roxana Reynoso for kindly providing the labelled hormones, PRL and LH, for radioimmunoassays, respectively. This work was supported by research Grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Universidad de Buenos Aires, and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References

1. Goering PL, Waalkes MP, Klaassen CD. Toxicology of cadmium. In: Goyer RA, Cherian MG (eds.) *Toxicology of metals: biochemical aspects, handbook of experimental pharmacology*. New York, NY: Springer, 1995, p.189–213.
2. Satarug S, et al. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicol Lett* 2003; 137: 65–83.
3. Koller LD. Cadmium. In: Zelikoff JT, Thomas PT (eds.) *Immunotoxicology of environmental and occupational metals*. London: Taylor and Francis, 1998, p.41–61.
4. Henson MC, Anderson MB. The effects of cadmium on placental endocrine function. *Recent Res Dev Endocrinol* 2000; 1: 37–47.
5. Bhattacharyya MH, Wilson AK, Rajan SS, Jonah M. Biochemical pathways in cadmium toxicity. In: Zalup RK, Koropatnick J (eds.) *Molecular biology and toxicology of metals* London: Taylor and Francis, 2000, p.1–74.
6. Massanyi P, et al. Effects of cadmium on ultrastructure and steroidogenesis in cultured porcine ovarian granulosa cells. *Acta Veterinaria* 2000; 69: 101–106.
7. Varga B, et al. Age dependent accumulation of cadmium in the human ovary. *Reprod Toxicol* 1993; 7: 225–228.
8. Zadorozhnaja TD, et al. Concentrations of arsenic, cadmium, copper, lead, mercury, and zinc in human placentas from two cities in Ukraine. *J Toxicol Environ Health* 2000; 61: 255–263.
9. Piasek M, Blanusa M, Kostial K, Laskey JW. Placental cadmium and progesterone concentrations in cigarette smokers. *Reprod Toxicol* 2001; 15: 673–681.
10. Fiala J, et al. Is environmental cadmium a serious hazard to Czech population? *Int J Occup Med Environ Health* 2001; 14: 185–188.
11. Paksy K, et al. Effect of cadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells. *J Appl Toxicol* 1997; 17: 321–327.
12. Satoh M, et al. Perspectives on cadmium research. *Tohoku J Exp Med* 2002; 196: 23–32.
13. Leret M, Millan J, Antonio M. Perinatal exposure to lead and cadmium affects anxiety-like behaviour. *Toxicology* 2003; 186: 125–130.
14. Viaene MK, et al. Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study. *Occup Environ Med* 2000; 57: 19–27.
15. Poliandri A, et al. In vivo protective effect of melatonin on cadmium-induced changes in redox balance and gene expression in rat hypothalamus and anterior pituitary. *J Pineal Res* 2006; 41: 238–246.
16. Henson M, Chedrese P. Endocrine disruption by cadmium, a common environmental toxicant with paradoxical effects on reproduction. *Exp Biol Med* (Maywood, NJ) 2004; 229: 383–392.
17. Lafuente A, Cano P, Esquifino A. Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? *Biometals* 2003; 16: 243–250.
18. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis: a review. *Toxicology* 2003; 192: 95–117.
19. Poliandri A, et al. Cadmium induces apoptosis in anterior pituitary cells that can be reversed by treatment with antioxidants. *Toxicol Appl Pharmacol* 2003; 190: 17–24.
20. Ognjanović B, et al. Effect of chronic cadmium exposure on antioxidant defense system in some tissues of

rats: protective effect of selenium. *Physiol Res* 2008; 57: 403–411.
21. Amara S, et al. Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. *J Reprod Dev* 2008; 54: 129–134.
22. Galbraith R. Heme oxygenase: who needs it? *Proc Soc Exp Biol Med* 1999; 222: 299–305.
23. Nath R, et al. Molecular aspects, physiological function, and clinical significance of metallothioneins. *Crit Rev Food Sci Nutr* 1988; 27: 41–85.
24. Ebadi M. The antioxidant properties of zinc and metallothionein. *Neurochem Int* 1996; 29: 159–166.
25. Vallee B. The function of metallothionein. *Neurochem Int* 1995; 27: 23–33.
26. Uchida Y, et al. The growth inhibitory factor that is deficient in the Alzheimer's disease brain is a 68 amino acid metallothionein-like protein. *Neuron* 1991; 7: 337–347.
27. Garrett S, et al. Expression of MT-3 protein in the human kidney. *Toxicol Lett* 1999; 105: 207–114.
28. Hozumi I, et al. Metallothionein-3 is expressed in the brain and various peripheral organs of the rat. *Neurosci Lett* 2008; 438: 54–58.
29. Quaife C, et al. Induction of a new metallothionein isoform (MT-IV) occurs during differentiation of stratified squamous epithelia. *Biochemistry* 1994; 33: 7250–7259.
30. Poliandri A, et al. Nitric oxide protects anterior pituitary cells from cadmium-induced apoptosis. *Free Radical Biol Med* 2004; 37: 1463–1471.
31. Tian L, Lawrence DA. Metal-induced modulation of nitric oxide production in vitro by murine macrophages: lead, nickel, and cobalt utilize different mechanisms. *Toxicol Appl Pharmacol* 1996; 141: 540–547.
32. Katakai K, Liu J, Nakajima K, Keefer LK, Waalkes MP. Nitric oxide induces metallothionein (MT) gene expression apparently by displacing zinc bound to MT. *Toxicol Lett* 2001; 119: 103–108.
33. Itoh N, et al. Reduced bactericidal activity and nitric oxide production in metallothionein-deficient macrophages in response to lipopolysaccharide stimulation. *Toxicology* 2005; 216: 188–196.
34. Rao M, Chhunchha B. Protective role of melatonin against the mercury induced oxidative stress in the rat thyroid. *Food Chem Toxicol* 2009 [Epub ahead of print].
35. Reiter RJ, Tan DX, Manchester LC. Melatonin: detoxification of oxygen and nitrogen-based toxic reactants. *Adv Exp Med Biol* 2003; 527: 539–548.
36. Hardeland R, Poeggeler B, Niebergall R. Oxidation of melatonin by carbonate radicals and chemiluminescence emitted during pyrrole ring cleavage. *J Pineal Res* 2003; 34: 17–25.
37. Guenther A, Schmidt S, Laatsch H. Reactions of the melatonin metabolite AMK (N1-acetyl-5-methoxykynuramine) with reactive nitrogen species: formation of novel compounds, 3-acetamidomethyl-6-methoxycinnolinone and 3-nitro-AMK. *J Pineal Res* 2005; 39: 251–252.
38. Kumar P, et al. Ascorbic acid, garlic extract and taurine alleviate cadmium-induced oxidative stress in freshwater catfish (Clarias batrachus). *Sci Total Environ* 2009; 407: 5024–5030.
39. Messaoudi I, et al. Reversal of cadmium-induced oxidative stress in rat erythrocytes by selenium, zinc or their combination. *Exp Toxicol Pathol* 2009, [Epub ahead of print].
40. Lee K, Lau K, Ho S. Effects of cadmium on metallothionein-I and metallothionein-II mRNA expression in rat ventral, lateral, and dorsal prostatic lobes: quantification by competitive RT–PCR. *Toxicol Appl Pharmacol* 1999; 154: 20–27.
41. Niswender G, et al. Radioimmunoassay for rat prolactin. *Proc Soc Exp Biol Med* 1969; 130: 793–797.
42. Nordberg G, Jin T, Nordberg M. Sub-cellular targets of cadmium nephrotoxicity: cadmium binding to renal membrane proteins in animals with or without protective metallothionein synthesis. *Environ Health Perspect* 1994; 102: 191–194.
43. Smalinskiene A, et al. Estimation of the combined effect of *Eleutherococcus senticosus* extract and cadmium on liver cells. *Ann NY Acad Sci* 2009; 1171: 314–320.
44. Urani C, et al. Metallothionein and hsp70 expression in HepG2 cells after prolonged cadmium exposure. *Toxicology In Vitro* 2007; 21: 314–319.
45. Miller J, Boswell F. Cadmium, lead and zinc in growing rats fed corn leaf tissue grown on soil amended with sewage sludge or heavy metal salts. *Environ Health Perspect* 1981; 42: 197–202.
46. Pillai A, Priya L, Gupta S. Effects of combined exposure to lead and cadmium on the hypothalamic–pituitary axis function in proestrous rats. *Food Chem Toxicol* 2003; 41: 379–384.
47. Lafuente A, Márquez N, Pazo D, Esquifino AI. Cadmium effects on dopamine turnover and plasma levels of prolactin, GH and ACTH. *J Physiol Biochem* 2001; 57: 231–236.
48. Oishi Y, et al. Cellular proliferation in the anterior pituitary gland of normal adult rats: influences of sex, estrous cycle, and circadian change. *Anat Record 1993*; 235: 111–120.
49. Post J, Hoffman J. Cell renewal patterns. *New Eng J Med* 1968; 279: 248–258.