

Chapter 8

**CADMIUM IN THE WALL LIZARD *PODARCIS SICULA*:
MORPHOLOGICAL AND MOLECULAR EFFECTS ON
EMBRYONIC AND ADULT TISSUES**

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ABSTRACT

Cadmium is a persistent contaminant accumulated in the environment from both anthropogenic and natural sources. Every year, large quantities of this metal are released in the different environmental compartments and may pose a significant threat to the biota exposed. Intracellular damage caused by cadmium exposure includes protein denaturation, lipid peroxidation, generation of reactive oxygen species and DNA strand breaks. Many studies have also demonstrated that this ion has a teratogenic or lethal effect on embryos, related to the dose and exposure time. In spite of the wide number of studies carried out in laboratory mammals, data on cadmium effects on fertility, reproduction and embryonic development of wild terrestrial vertebrates are still limited. In particular, information on the consequences of environmental cadmium exposure on reptiles survival and biodiversity are particularly scanty. Reptiles are presently considered highly susceptible to a number of environmental pollutants and this has contributed to the global decline of several wild populations of turtles, crocodylians and lizards. As regarding cadmium effects on offspring survival, reptiles eggs for a long time have been considered well protect from the external environment and the presence of environmental contaminants in eggs or developing embryos has been attributed to a maternal transference during vitellogenesis and oviductal egg retention. More recently, it has been demonstrate that metal ions and organic contaminants present in soil may cross the flexible parchment-like shell of reptilian eggs. In consideration of the few data currently available we decided to investigate cadmium effects on biological processes such as reproduction and development in the reptile *Podarcis sicula*, a lizard species

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inhabiting both pristine and urbanized areas. The results summarized in this chapter clearly demonstrate that cadmium can interfere with the welfare and the reproductive fitness of adults, and with the development and survival of embryos. In turn, these detrimental effects on offspring production may dramatically modify the survival of wild populations inhabiting contaminated areas significantly endangering the local biodiversity and the ecological equilibrium.

INTRODUCTION

Cadmium (Cd) is a non-essential toxic heavy metal with toxicity many times higher than that of many other heavy metals (Beyersmann and Hartwig, 2008). Used primarily in metal coatings and nickel-cadmium batteries, with worldwide industrialization Cd pollution has dramatically increased due to its wide use in many other industrial operations including pigments, metal coatings and plastics. So, for many years Cd has been constantly introduced into the atmosphere, water and soil as a result of the smelting of ores, burning of fossil fuels, waste incineration, urban traffic and as a by-product of phosphate fertilizers (Thornton, 1992). The primary routes of Cd exposure in terrestrial animals are via inhalation and ingestion of Cd contaminated food (Kliment, 1996; Nam and Lee, 2006). As non essential trace element, Cd may cause toxicity by disturbing the cellular homeostasis of essential metal ions, such as copper, zinc, and calcium. Cadmium has a high affinity for zinc- and calcium-binding sites and can displace these metals from pre-existing complexes (Predki and Sarkar, 1994). Proposed mechanisms by which Cd induces damage involve metal binding to cysteine residues and the generation of reactive oxygen species (Risso-de Faverney et al., 2001; Pokrovsky et al., 2008). Genetic analyses on both plant and animal species have demonstrated that the exposure to sub-lethal Cd concentrations results in identifiable changes in gene expression (Carginale et al., 2002; Liao and Freedman, 2002; Minglin et al., 2005). Expression profiling analyses successfully aided in the discovery of genes regulated in response to Cd exposure, including the important metal biomarker metallothionein and other important stress responsive proteins such as heat shock proteins (Carginale et al., 1998; Bertin and Averbeck, 2006; Luparello et al., 2007). Cadmium induces overexpression of the proto-oncogenes *c-fos*, *c-myc* and *c-jun*, and of some translation factors (Bertin and Averbeck, 2006). Several groups have also demonstrated that Cd-induced cell death is associated with increased p53 mRNA levels in different cell lines after exposure to Cd (Lag et al., 2002). Cadmium ions interfere on reproductive endocrinology modifying the expression of many sex hormones (Henson and Chedrese, 2004). Exposure of rodents to the metal results in a down-regulation of pituitary hormones, including gonadotropins, prolactin, ACTH, growth hormone, and thyroid-stimulating hormone (Lafuente et al, 2003). Similarly, in pseudopregnant rats and in cultured granulosa cells from both rats and humans, Cd inhibits progesterone synthesis (Piasek et al., 2002; Zhang and Jia, 2007). Moreover, many other genes are regulated by the presence of Cd; some of these have been identified even though their function is unknown, others are yet to be highlighted (Carginale et al., 2002; Minglin et al., 2005).

Identifying these genes will help the understanding of the molecular mechanisms underlying the cellular response to Cd. For a long time, the buffering capacity of the soil has been considered able to limit the accumulation of Cd. As a result, research has focused on the

contamination of aquatic environments, neglecting terrestrial wildlife. However, recent data have revealed that heavy metals accumulate in the soil by changing its composition. It has been demonstrated that Cd concentration in the topsoil is highly variable ranging from 0.10 to over 600 mg/kg in the main polluted areas (Stafilov et al., 2010).

As a consequence, parallel to the awareness of the soil pollution problem, the scientific community is setting up a growing number of projects aimed to investigate the effects of Cd on organisms inhabiting the soil, especially plants and invertebrates (Verbrugger et al., 2009; Peralta-Videa et al., 2009; DalCorso et al., 2008; Veltman et al., 2008; Chabicovsky et al., 2004; Kammenga et al., 2000). Up today, in spite of the wide numbers of standardized protocols available for measuring the effects of soil Cd contamination to invertebrates (Løkke and van Gestel, 1998; Dallinger et al., 2004), data for vertebrates are still limited. Reptiles are presently considered susceptible to a number of factors, which have contributed to the global decline of several reptile species like turtles, crocodylians or lizards (Gibbons et al., 2000). Environmental pollution is one of the main threats affecting the conservation of reptile populations, and some reptile species have been identified as good bioindicators of pollution in their environments thanks to their persistence in a variety of habitats, wide geographic distribution, longevity and site fidelity (Lambert, 1997; Crain and Guillete, 1998).

The common wall lizard *Podarcis sicula* is widely distributed in Italy, where is frequently present in urbanized and cultivated areas. Throughout the life stages of this lizard, the contact with the soil may be a potential source of contaminant exposure and begins in ovo when eggs are deposited in soil. Thus, exposure may already take place earlier, during embryonic development. At oviposition, lizard eggs contain insufficient water to complete development and absorb large amounts of water from the surrounding soil (Marco et al., 2004a; Sexton et al., 2005).

Contaminants that are capable of permeating and crossing the parchment-like shell may interfere or disrupt normal physiological functions in the developing embryo, including regulation of gene expression. In consideration of the data currently available and in light of the potentially serious consequences of environmental Cd exposure to reptiles conservation and biodiversity, we have studied the uptake and the effect of Cd in the tissues of the lizard *Podarcis sicula* and, in particular, in liver and ovary. We have also investigated Cd effects on oocyte recruitment and on embryo development, analysing the gene expression profiles in lizard embryos following in ovo Cd exposure.

MATERIALS AND METHODS

Animals

Adult specimens of *Podarcis sicula* of field origin were caught in the outskirts of Naples. The animals were kept in a terrarium and maintained under conditions of natural temperature and photoperiod. Embryos were obtained by collecting freshly laid eggs from gravid females. All the experiments were carried out in compliance with the ethical provisions enforced by the European Union and authorized by the National Committee of the Italian Ministry of Health on in vivo experimentation (Dpt. for Veterinary Public Health, Nutrition and Food Safety).

Cadmium Treatments

For the treatments on adults, the animals were fed every second day for 60 days with 1 µg CdCl₂ per g of body weight; each dose of Cd consisted in 40 µl of an appropriate solution of CdCl₂ delivered directly into the mouth of the animals with a Gilson micropipette. The relative control group received 40 µl of tap water at the same time intervals. The animals were sacrificed after 10, 30 and 60 days of treatment. For in ovo treatments, eggs collected from a single clutch were divided in two different terrariums, one containing natural soil and another containing soil contaminated with a solution of CdCl₂ for a final concentration of Cd 50mg/kg soil. Terrariums were maintained at natural temperature and water lost as vapour was reintroduced by daily soil nebulisations with tap water. After 20 days from deposition, embryos were recovered from shells and immediately dipped in RNAlater to prevent RNA degradation.

Cadmium Content in Lizard Tissues

The determination of total Cd content in *P. sicula* tissues was obtained by using the atomic absorption spectroscopy (AAS). Tissues were digested at 70 °C with 65% HNO₃ (Ultrapure, Fluka), using 1 ml of acid every 50 mg of wet tissue. Cadmium content in the supernatant obtained after centrifugation for 5 min at 12.000xg was determined by the graphite furnace method, using a Varian atomic spectrometer AA200.

Cytological Observations

For the light microscopy analyses, adult tissues and embryos were fixed in Bouin's solution and processed for paraffin wax embedding according to routine protocols. Sections were stained with haematoxylin-eosin or Mallory's trichrome to show general morphology.

Other sections were stained with periodic acid/Schiff (PAS) to detect glycogen and with specific lectins (WGA, *Triticum vulgare*, wheat germ; LEA, *Lycopersicon esculentum* agglutinin, tomato; DBA, *Dolichos biflorus*, horse gram; PNA, *Arachis hypogaea*, peanut agglutinin) to detect glucid residues. Stainings were performed as previously described (Simoniello et al., 2010). Tunel staining was carried out using the In Situ Apoptosis Detection Kit (Upstate Biotechnology Inc) following the manufacturer's instructions.

For the transmission electron microscopy analysis, small blocks of liver were fixed according to Karnovsky (1965), dehydrated in ethanol, and embedded in Epon 812 following the manufacturer's instructions. Ultrathin sections were double stained with uranyl acetate and lead citrate.

Gene Expression Profiling

The mRNA differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) analysis was applied to obtain insight into the gene expression changes induced in the

embryo by Cd exposure. DNA-free total RNA extracted from either control or Cd-treated embryos was reverse-transcribed using a set of three, one base anchored oligo(dT) primers (H-T11A/C/G). Amplification of cDNA fragments was performed using combinations of the anchored H-T11 primers and 28 different upstream primers in the presence of the radiolabelled nucleotide [α - 33 P]dATP. A total of 320 separate PCR reactions were generated using the two different pools of cDNA amplified with about 80 combinations of primers. Amplification products were resolved by denaturing polyacrylamide gel electrophoresis. Autoradiography of dried gels allowed the identification of Cd-responsive fragments that were recovered from the gels, reamplified, inserted into a pCRII-TOPO vector, cloned in *Escherichia coli* and sequenced bidirectionally (Primm Biotech). The homology search of genes was performed by FASTA and BLAST analyses. Reverse Northern dot-blot (Zhang et al., 1996) was then performed to screen for cDNA fragments that truly represented differentially expressed mRNAs.

RESULTS

Cadmium Effects in Adult Tissues

Total Cd content in gut, liver, kidney, ovary and brain from adult specimens of *Podarcis sicula* was determined by AAS. To better estimate the effect of individual variability on data dispersion, Cd accumulation in each organ has been represented by box plot.

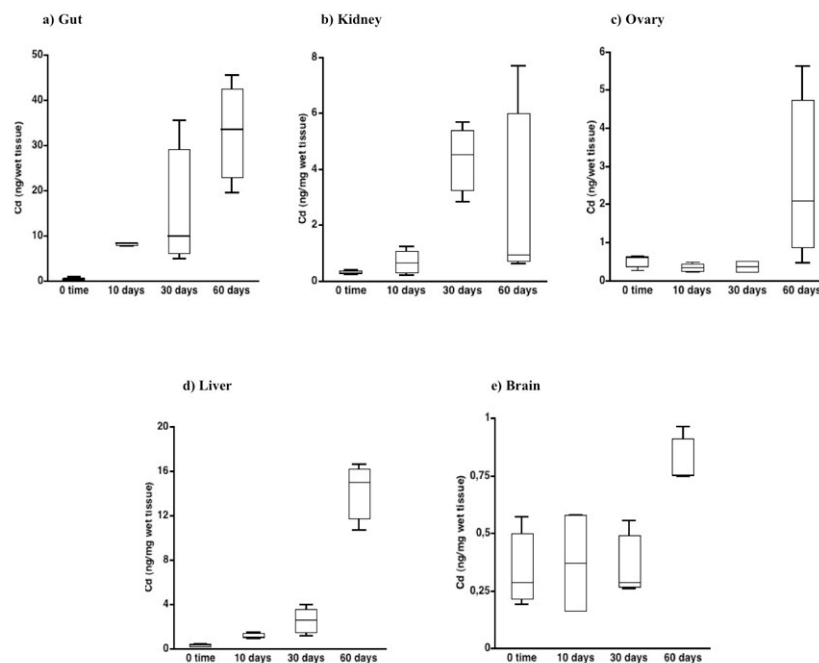


Figure 1. Graphical representation by box plots of Cd accumulation in lizard tissues after chronic Cd treatments. The exposure was obtained feeding animals every second day for 60 days with 1 μ g Cd per g of body mass; Cd content was determined by atomic absorption spectrophotometry at day 10, 30 and 60 from starting treatment.

The presence of detectable amounts of Cd in tissues of untreated animals can be due to the urbanized sites of capture of wild specimens thus demonstrating the presence of a detectable environmental contamination.

Box plots of the dietary Cd absorption (Figure 1) show that Cd levels increase with the exposure time; Cd begins to accumulate in gut (Figure 1a) and then, probably via passive diffusion or H(+)-antiport as in mammalian cells (Endo et al. 2000), is secreted by intestinal cells and reaches other tissues (Figure 1b-e). Although a large body of evidence demonstrates that in chronic Cd intoxication kidney is the main target organ (Min et al., 1996; Liu et al., 2000; Sabolic et al., 2001), we find that, at the end of the treatment, Cd concentration is twofold higher in liver (Figure 1d) than in kidney (Figure 1b). A prolonged dietary Cd exposure brings about an increase in Cd concentration in ovary (Figure 1c) and in brain (Figure 1e), albeit in the latter at a lower level with respect to other organs. These data are at variance with previous observations according to which the blood-brain barrier is capable to prevent Cd entrance into the central nervous system and the neurotoxic effects of Cd are probably due to an interference of Cd with zinc metabolism, rather than to a direct effect of the metal on brain cells (Jin et al., 1998).

Hence, AAS data indicate that in *Podarcis* the liver sequesters Cd ingested by food and that this organ is a main target of heavy metal contamination. Cytological analyses demonstrate that Cd induced injury is fast, with toxic effects already evident 48 hours after the beginning of administration. Treated animals show similar liver alterations maintained until the end of the experiments. These alterations are summarized in Table 1.

The most recognisable changes are vascularization and oedema with groups of red blood cells in the parenchyma and sinusoids markedly enlarged. The hepatocytes are profoundly altered with cytoplasm clearly less dense than in controls suggesting hydropic swelling. Transmission electron microscopy confirms this evidence showing treated hepatocytes highly vacuolated with cytoplasm and organelles clustered much closer to the nucleus. In liver Cd also induces remarkable changes in glycoconjugates content.

The cytoplasm is apparently poor in glycogen. PAS staining of sections reveals a decreased affinity in the hepatocyte cytoplasm thus indicating an altered sugar metabolism; lectin staining demonstrates a decrease in cytoplasmic glycogen and D-N-acetyl-glucosamine (glcNAc)₂ and an increase in N-acetyl-glucosamine (glcNAc)_{3a}.

Table 1. Main Cd induced changes in liver of the lizard *Podarcis sicula*

Cadmium effects on liver	
MORPHOLOGY	Hepatocytes swelling Vascularization Oedema
LIPID METABOLISM	Formation of hydrophobic vesicles
SUGAR METABOLISM	Alteration of glycoconjugates: Decreased PAS positivity in cytoplasm Increased WGA, PNA, DBA staining Decreased LEA staining

Table 2. Main Cd induced changes in ovary of the lizard *Podarcis sicula*

Cadmium effects on ovary
Degeneration of oocytes
Atretic early previtellogenic follicles
Alteration in follicle organization
Alteration in epithelium and zona pellucida

On the contrary, Cd does not alter liver cell proliferation and death: no evidence of proliferation or apoptosis is seen in both control and Cd-treated hepatocytes. TUNEL staining shows occasional Kupffer cells and monocytes with labelled nuclei. The localization of caspase 3 retrieves the same result with rare Kupffer cells and monocytes labelled. The wide-ranging Cd effects observed in the liver are likely to affect function and cycle of cells. On this basis we would predict a plethora of effects on many physiological processes in which the liver plays a role; oogenesis, for example, could be impaired as functional alteration in the liver may affect the synthesis of vitellogenin, a glycolipoprotein that is produced in the liver and stored in the oocyte to serve as reserve material for the embryo (Roy and Chatterjee, 1983). This may lead to a loss of reproductive performance that could have significant effects on population survival. A dramatic loss of reproductive performance is also suggested by cytological observations on Cd-treated ovaries (Table 2). Germinal beds are particularly large and rich in prefollicular oocytes as compared to controls. The presence of several pycnotic nuclei suggests that zygotene-diplotene oocytes are undergoing a massive degeneration. Degenerative events are also evident in several previtellogenic follicles in which atretic oocytes and/or apoptotic follicle cells can be observed. It is significant that in these stages, in control gonads, atresia is a very rare event: in *Podarcis*, in fact, oocyte selection occurs exclusively in prefollicular stages (Andreuccetti et al., 1990).

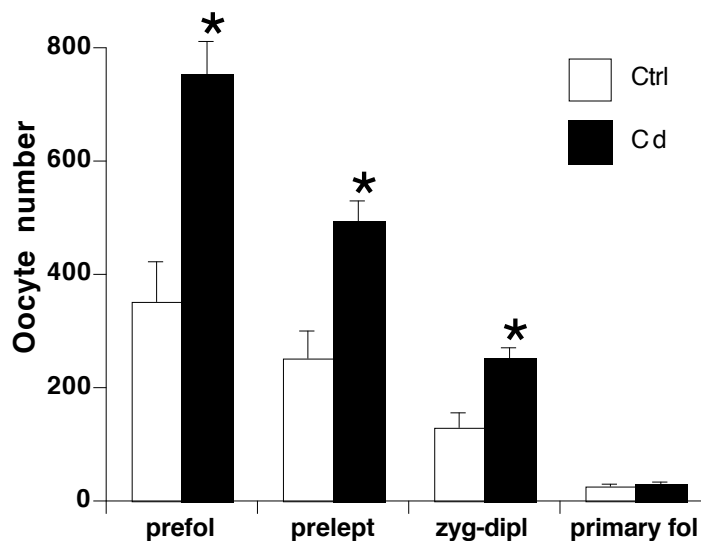


Figure 2. Changes in oocytes number after Cd treatments. Statistical significance (*= $p < 0.05$) was assessed by Anova. Stage legend: prefol, prefollicular; prelept, preleptotene; zyg-dipl, zygotene-diplotene; primary fol, primary follicle.

Counting of germ cells in the germinal beds and in the different stages of oogenesis (Figure 2) confirms that Cd treatment increases about twofold the total number of prefollicular, preleptotene and zygotene-diplotene oocytes. Investigations on follicle recruitment indicate that the supernumerary oocytes formed after Cd treatment become apoptotic so that the number of primary follicles does not increase (Figure 2). The formation of extra oocytes would activate the endogenous mechanism controlling germ cell number, otherwise Cd might have a direct pro-apoptotic effects on zygotene-diplotene oocytes as reported in other species (Kitana and Callard, 2008).

Another interesting aspect emerging from results is that Cd induces follicular atresia. In *Podarcis*, this is a very rare event since oocyte selection occurs between the zygotene stage and the time the primary follicle organizes (Andreuccetti et al., 1990). The presence of atretic follicles suggests that the metal may act on ovaries reducing female fecundity; our data showing a relevant reduction in clutch size support this conclusion.

Cadmium Effects on Embryos

Data described in this chapter clearly demonstrate that Cd may cross through the flexible shell of *P. sicula* eggs. Although no mortality is observed in ovo exposed embryos, they show significant morphological alterations in encephalon and eyes (Table 3). Fine histological analyses confirm severe Cd-induced malformations in the bone structure (including the lack of the cranial vault, the deformation of the skull base, the palate, the otic capsule and jaws), in the encephalic areas and in the eye. In particular, in the brain, more severe malformations occur in telencephalon, diencephalon and mesencephalon. No alterations are observed in medulla oblongata, spinal cord and visceral organs, whose structures are always comparable to those of controls throughout development.

Gene expression profiling analysis by DDRT-PCR demonstrates that Cd intake is able to modify the expression of at least 14 embryonic genes; in particular, 9 genes are up regulated and 5 genes are down regulated. The nucleotide sequences of the Cd-regulated cDNAs have been determined and compared with those in GenBank Nucleotide Sequence Databases through the NCBI server, to identify putative proteins encoded by these mRNAs.

Table 3. Main morphological abnormalities in *Podarcis sicula* embryos incubated in Cd contaminated soil

Morphological alterations	Affected embryos
Anencephaly	5%
Telencephalon and diencephalon abnormalities (exencephaly, ventricle swelling)	60%
Mesencephalon abnormalities (exencephaly, ventricle swelling)	70%
Eye abnormalities (microphthalmia, retinal malformations)	50%
Facial abnormalities	20%
Limb abnormalities	1%
Trunk abnormalities	0%

Table 4. Sequence analysis of differentially expressed mRNAs in *Podarcis sicula* embryos following Cd exposure

DDproduct	Cd-effect	Sequence homology	E-value
DD1	–	No significant hits found	–
DD2	+	No significant hits found	–
DD3	+	No significant hits found	–
DD4	+	No significant hits found	–
DD5	–	Novel protein from <i>Danio rerio</i> DNA	2e ⁻⁸
DD6	+	Novel protein from <i>Danio rerio</i> DNA	1e ⁻⁶
DD7	–	<i>Xenopus tropicalis</i> EST	5e ⁻¹⁴
DD8	+	EST from cold-stressed <i>Descurainia sophia</i> 1-month seedlings cDNA	1e ⁻⁵
DD9	–	<i>Gallus gallus</i> HMGR mRNA for 3-hydroxy-3-methylglutaryl-CoA	1e ⁻⁴
DD10	+	<i>Cynops pyrrhogaster</i> Voltage-dependent sodium channel mRNA	4.5e ⁻⁹
DD11	+	<i>Rana catesbeiana</i> GABA-B receptor R2 subunit	5e ⁻¹²
DD12	+	<i>Homo sapiens</i> Development and differentiation-enhancing factor 1	1e ⁻¹⁴
DD13	+	<i>Gallus gallus</i> putative uncharacterized protein with a BTB/POZ	2e ⁻¹⁹
DD14	–	<i>Xenopus tropicalis</i> Basic transcription factor 3 (BTF3)	3e ⁻⁶

The results of NCBI BLAST analyses allow us to identify 10 different genes, which show significant identities to translated sequences contained in the GenBank database, whereas the other 4 cDNA clones are not significantly homologous to any translated sequence, suggesting that they may either encode unidentified proteins, or correspond to untranslated non conserved regions of mRNAs. Table 4 summarizes the results of the sequence analysis of Cd responsive cDNAs.

Among the 10 cDNA fragments identified, 2 encode putative proteins with unknown function, 2 fragments show significant homologies with Expressed Sequence Tags (EST) and the other 6 are assigned to 6 different putative functions. In particular, the identified proteins encoded by the down-regulated genes are the 3-hydroxy-3-methylglutaryl-CoA reductase and the basic transcription factor 3, whereas the identified proteins encoded by the up-regulated genes are: the voltage-dependent sodium channel, the GABA-B receptor R2 subunit, the development and differentiation enhancing factor 1 and a putative BTB (POZ) domain-containing protein. The profiles of gene expression suggest that pathways associated with membrane trafficking, protein-protein interactions, neuronal transmission and gene regulation are affected by exposure to a sublethal dose of Cd.

Membrane trafficking and protein-protein interactions. The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is involved in the synthesis of cholesterol, an essential structural component of vertebrate cell membranes, where it is required to establish proper membrane permeability and fluidity. Cholesterol plays a vital role during vertebrate

development: loss of HMGR results in early embryonic lethality in mouse models (Ohashi et al., 2003). Cholesterol, in fact, is the precursor of steroid hormones and glucocorticoids; membrane cholesterol also affects the activity of growth factor receptors and cell-adhesion molecules by clustering these cell surface proteins into lipid rafts (Michel and Bakovic, 2007).

The development and differentiation-enhancing factor 1 (DDEF1) encodes a product that shared structural features with centaurin-family proteins, and encompasses an ADP-ribosylation factor GTPase-activating protein (ArfGAP) domain and two ankyrin repeats. The ankyrin repeat is a protein-protein interaction motif that occurs in a large number of functionally diverse proteins such as transcriptional initiators, cell-cycle regulators, cytoskeletal and signal transducers (Voronin and Kiseleva, 2007). Centaurins regulate cyclic activation of Arfs, GTP-binding proteins that function in vesicle formation, intracellular vesicle trafficking and in signal transduction networks playing a more direct role in cytoskeletal organization and progression through mitosis (Randazzo and Hirsch, 2004). An overexpression of an ADP-ribosylation factor was found in Cd-exposed *Brassica juncea* (Minglin et al., 2005). It is known that in plants ARF stimulation is associated to oxidative stress, osmotic stress and plant defence (Zuk et al., 2003). In addition, it has been demonstrated that a member of the centaurins family contributes to Cd resistance in fission yeast, probably by maintaining membrane integrity and by modulating membrane trafficking (Vashisht et al., 2009). Therefore, it is possible that ARFs may play a regulatory role in coping with multi-stresses imposed by Cd uptake in animals as in plants.

Neuronal transmission. The expression of genes encoding the voltage-dependent sodium channel and the GABA-B receptor R2 subunit are Cd-activated.

The voltage-gated sodium channels are typical of excitable cells such as neurons, myocytes, and certain types of glia. Density and activity of these channels are fundamental in controlling nerve transmission, sensory transduction, muscle contraction, synaptic plasticity and neuronal circuit formation (Catterall et al., 2005). Up- and down-regulations of multiple isoforms of sodium channel molecules occur in response to various therapeutic drugs and noxious insults, which may be the basis for adaptive and maladaptive neuronal remodelling. Like Cd, the antiepileptic valproic acid (VPA) drug induces the up-regulation of the gene expression, probably activating the calcium channel and the catecholamine secretion (Yamamoto et al., 1997). Therapy with VPA during early pregnancy can cause exencephaly in mammals (Ogawa et al., 2007); interestingly, a similar damage is found in Cd-treated *Podarcis* embryos, suggesting a shared mechanism.

The metabotropic GABA-B receptors are widely distributed within the central nervous system and also in peripheral autonomic terminals. During mammalian development, GABA-B receptor signalling is crucial for the modulation of nascent and mature synapses and for developmental processes such as neuronal migration and axon growth (Kornau, 2006). Again, VPA leads to an increased activity on GABA-B receptors (Pranzatelli and Nadi, 1995), sharing with the Cd ions the same final effects on nervous system.

Gene regulation. The BTB/POZ domain is an evolutionarily conserved protein-protein interaction motif (Albagli et al. 1995). The BTB domain is found in a variety of proteins including actin-binding proteins and many transcriptional regulators that usually contain zinc-finger binding motifs. Many BTB proteins are transcriptional regulators that mediate gene expression through the control of chromatin conformation (Qi et al., 2006). The functions of several BTB genes are required for normal *Drosophila* eye development (Wen et al., 2000).

Likely the modifications in the expression of BTB/POZ domain genes might elicit changes in the expression of many genes, whose effects are difficult to predict.

The basic transcription factor 3 (BTF3) is involved in the initiation of transcription by RNA polymerase from proximal promoter elements such as the TATA box and CAAT box sequences (Zheng et al., 1987). BTF3 loss-of-function mutation in mice leads to death in the early stages of development pointing to an important role of this gene during normal development (Deng and Behringer, 1995). In addition, BTF3 is also involved in cell cycle regulation and apoptosis (Thiede et al., 2001).

CONCLUSION

Many animal species are under extinction risk due to several factors, such as habitat loss, global warming, introduction of exotic species, wildlife trade and emerging pathologies. Environmental pollution is often invoked as one of the most relevant extinction causes for amphibians, whereas terrestrial vertebrates such as reptiles and birds for long time have been considered more resistant to soil contaminants. Furthermore, their embryos have been considered well protected by shells from the external environment; the presence of environmental contaminants in eggs or developing embryos has been attributed to maternal transfer during vitellogenesis and oviductal egg retention (Guirlet et al., 2008). More recently though, it has been demonstrated that metalloids and organic contaminants present in soil may cross the parchment-like shell of reptilian eggs (Marco et al., 2004a, 2004b; Gómara et al., 2007).

Data described in this chapter clearly demonstrate that both adults and embryos of the lizard *Podarcis sicula* are highly susceptible to Cd effects. In adults, oral Cd administration, consistent with a transfer via food chain, gives rise to the accumulation of the metal in various tissues than gut. The accumulation in liver is accompanied by severe damage at multiple levels interfering with tissue structure and function; in ovary Cd accumulation is accompanied by stimulation of oogonial proliferation, oocyte recruitment and follicular atresia. The effects exerted by Cd in *Podarcis* ovary seem to be not typically estrogenic as expected (Byrne et al., 2009) but rather FSH-like. All together, these effects strongly reduce fecundity and reproductive performance.

In embryos, incubation in Cd contaminated soil causes damage to head skeletal structures and to forebrain vesicles. In addition, Cd present in the soil is able to interfere with gene regulation; in particular, Cd affects the expression of genes involved in molecular pathways associated with membrane trafficking, signal transduction, neuronal transmission and regulation of gene transcription. The dysregulation in the expression of these genes could explain many of the morphological alterations displayed by lizard embryos. Indeed, similar damage (in particular, anencephaly and eye malformations) can be observed in mammalian and amphibian embryos exposed to the valproic acid, an antiepileptic drug capable of altering the expression of these same Cd responsive genes (Menegola et al., 1996; Pennati et al., 2001; Massa et al., 2005). Further studies are needed to determine if a correlation exists between Cd induced morphological abnormalities and the changes in the expression of specific Cd responsive genes.

Although no in ovo mortality was observed, the severe malformations of embryos incubated in Cd contaminated soil may be incompatible with adult survival. Since almost all reptilian species lay their eggs in subterranean nests, the rapid decline in many reptilian populations observed in recent years might be partly ascribed to developmental failures in offspring in these increasingly polluted environments, and partly ascribed to the impaired fecundity of Cd contaminated females, leading to a loss of reproductive performance and a likely decline in the number of individuals for wild populations living in polluted areas.

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