Cadmium, organ toxicity and therapeutic approaches. A review on brain, kidney and testis damage.

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Cadmium, organ toxicity and therapeutic approaches. A review on brain, kidney and testis damage.

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Running title: Cadmium in brain, kidney and testis.

Abstract

Background: Cadmium (Cd) is a highly diffused heavy metal and, therefore, a ubiquitous environmental toxicant. For the general population, the principal causes of exposure to Cd are considered cigarette smoking, air pollution and consumption of contaminated water and foods, while an occupational exposure usually involves human during mining or manufacturing of batteries and pigments that utilize Cd.

Methods: We undertook a structured search in literature about Cd. This metal is noxious on the cells of many organs, among which the kidney, the testis and the brain will be considered in this review.

Results: The toxic effects induced by Cd include many specific mechanisms, such as the oxidative stress, cellular death and inflammation. As no specific therapy for the prevention or treatment of the morbidity and mortality associated with Cd exposure is available, the state of the art of the therapeutic approaches is illustrated.

Conclusion: Nowadays, a therapy able to counteract Cd toxicity is still lacking and the development of new therapeutic agents is requested.

Keywords, Cadmium, toxicity, kidney, brain, testis, therapeutic approaches.

1. Introduction

Environmental pollutants, among which many industrial and domestic chemicals, pesticides, fertilizers, heavy metals are included [1], represent serious hazard to human health [2]. In particular, heavy metals cause serious impact on the environment owing to their release from industries, agriculture and urban waste. Humans and animals are therefore exposed to toxic metals, which accumulate in the organism through many entry pathways, among which inhalation and ingestion are the most important [3] (Figure 1).

Cadmium (Cd) is a relatively rare metal, naturally occurring in its inorganic form. However, despite the efforts of the government of many countries to reduce its levels, anthropogenic sources have augmented Cd in atmosphere, soil, water and animals used in human feeding [4]. Furthermore, Cd is a not biodegradable element and possesses a biological half-life of 20-30 years, so that its concentration in the environment is progressively increasing [5].

It has been reported that the major sources of Cd are food, cigarette smoke and re-charged nickel-cadmium batteries. In particular, tobacco contains significant amounts of Cd absorbed by the soil, so that smoking is a major source of exposure among the general population [5]. In fact, small particles, fumes and cigarette smoke are able to exceed the upper airways defense systems and to reach the alveolar epithelium, where they are absorbed, thus transferring in the blood the toxicants. Similarly, dietary Cd penetrates in the body through the gastrointestinal tract, with different degree of absorption depending on the dose, the type and the frequency of exposure to the toxicant [5].

In the systemic circulation, Cd is initially bound to albumin in blood plasma and then taken from blood cells. Albumin-bound Cd is transported to the liver, where Cd is released owing to the degradation of the complex, thus inducing the synthesis of metallothionein (MT) [6].

Metallothionein (MT), a small cysteine-rich protein, is a stress protein that can also protect from Cd toxicity and oxidant stress; in fact, in vitro or in KO mice for MT-1 and MT-2 genes Cd toxicity and oxidative stress are higher [7]. MT is also a scavenger of hydroxyl radicals [8].

Once absorbed, Cd is rapidly cleared from the blood and concentrates in various tissues, especially in liver and kidneys.

The mechanism responsible for Cd-induced toxicity is particularly complex. Cd exerts toxicity on the cells of various systems and tissues, by affecting their function directly or indirectly [6]: as a consequence, degeneration or even carcinogenic effects of the cells can be observed [9].

Cd induces tissue injury through oxidative stress [10-12], DNA changes [13–15] and involvement of transport pathways [16–18]. In addition, it can induce impairment of mitochondrial function with trigger of apoptosis [19] and activation of the inflammatory cascade [20].

The toxic effect of Cd is evident in many organs, among which kidney, testis and brain will be considered in our review.

2. Cadmium toxicity in brain

Cd is able to induce neurotoxic effects either in vitro or in vivo. In fact, its damages were observed in vitro in cortical neurons [21, 22], in the optic nerve [23], in anterior pituitary cells [24], in trigeminal neurons [25], in glioma and neuroblastoma cells [26] and in nerve-glia cells cultures [27]. The experimental neurotoxic effects of Cd have been reported also in neonatal mouse [28] and in adult rat brain [29-33].

Several reports studied the effect of Cd accumulation in the brain as a whole or in specific regions.

In fact, in humans, Cd caused neurological disorders such as learning disabilities and hyperactivity in environmental exposed children [34], while neuropsychological disorders [35], amyotrophic lateral sclerosis [36], striatum damages [37], Parkinsonism [38], Parkinson's and Alzheimer's disease [22] were described in occupationally exposed subjects.

In particular, in these subjects, symptoms such as headache and decreased equilibrium, reducing of vasomotor functioning, peripheral neuropathy, learning disabilities and defects in attention have been shown [34, 39].

Once entered in the body, owing to its long life, Cd exerts toxic effects in brain [33], which primarily depend on its possibility to cross the blood brain barrier (BBB) [40]. Discordant data are present on this topic. In fact, while a high BBB permeability with consequent accumulation in the brain was described [21, 22], a low penetration of Cd through the BBB was also shown, particularly when its full organization is reached during the developmental processes, therefore, an age-dependent toxic effect of Cd on the brain was demonstrated [41]. Additionally, a high accumulation of Cd in the choroid plexus and in the ependymocytes was also shown [42]. As a consequence, Cd induces severe neurotoxicity (Figure 1) and alters the normal functioning of the neurotransmitters, such as dopamine, norepinephrine and serotonin [32].

The mechanism of Cd induced neurological damages is incompletely known. It determines oxidative stress through the production of reactive oxygen species (ROS), able to induce an increased lipid peroxidation, an alteration of calcium homeostasis and a damage of antioxidant defenses, resulting in DNA injury [31, 32].

Cd-induced oxidative stress is associated to inflammation [43]. Under these conditions, cyclooxygenase (COX)-2 expression and prostaglandin (PG)-E2 synthesis are increased in cerebrovascular endothelial cells and in mouse peritoneal macrophages, respectively [44, 45], the intercellular adhesion molecule-1 (ICAM-1) is up-regulated [46] through the activation of nuclear factor (NF)-kB [44]. In particular, PGE2 and PGF2α increase is linked to alterations in BBB during inflammation [47].

Another important mechanism demonstrated in Cd exposure is the activation of Extracellular regulated kinase (Erk)1/2, c-Jun N-terminal kinase (JNK) and/or p38 Mitogen Activated Protein Kinase (MAPK) able to switch apoptosis in numerous types of cells, among which neuronal cells are included [48, 49]. However, neuronal apoptosis was shown to be only partially correlated to the activation of Erk1/2 and JNK, but not of p38 [22, 50].

An important role in the Cd induced apoptotic processes in neuronal cells is also played by Akt/mammalian target of rapamycin (mTOR) pathway [51]. mTOR, a Ser/Thr kinase, controls cell proliferation, growth and survival [52] and is regulated by Akt. A peculiar role in MAPKs and mTOR signaling pathways activation in the neuronal cells is played by a prolonged elevation of intracellular free Ca2+ [53, 54].

3. Cadmium toxicity in kidney

Numerous studies have demonstrated that Cd, either *in vitro* or *in vivo* is able to induce kidney damages [55 - 58]. Chronic exposure to Cd was shown to be the cause of the itai-itai disease, which occurred endemically in a specific district of Japan [59] and demonstrated clinical signs of renal tubular dysfunction, such as proteinuria, glucosuria and aminoaciduria, irreversible at an advanced stage [55]. These symptoms are now considered typical of either occupational or environmental Cd exposure or of various experimental models [58, 60]. Cd exposure can also influence calcium metabolism, causing hypercalciuria and formation of kidney stones [61].

As above indicated, Cd is bound to MT. The Cd–MT complex is small enough to be freely filtered through the renal glomerulus and then taken up by the S1 and S2 segments of the proximal tubules [62, 63]. In this way, a selective accumulation of Cd in cells of the proximal tubules occurs [6], even if glomerular and distal tubular damages have been also described [64].

Damaged tubular cells are degraded by the lysosomes with consequent release of Cd [65]. In this way, ROS are generated, which destroy DNA, proteins and lipids, and activate signaling pathways able to induce cell apoptosis [66] (Figure 1).

In vial cells, cytosolic Cd indirectly produces ROS through the generation of different radicals, such as superoxide radical, nitrogen species including peroxinitrite, nitric oxide and hydroxyl radical, thus causing damage typical of oxidative stress. In this way, lipid peroxidation and damage on many transport proteins, including Na+/K+ ATPase is obtained [67].

Oxidative stress is able to induce also inflammation with increased production of proinflammatory cytokines leading to renal tissue damage [68 - 71]. In fact, ROS activate NF-κB that subsequently translocates to the nucleus, where it regulates the transcription of response genes encoding IL-6 and tumor necrosis factor (TNF)-α and increases the transcription of Macrophage migration Inhibitory Factor (MIF) gene and other target genes [72]. Furthermore, chronic administration of Cd to rat is associated to an increase of COX-2 expression [65].

Cd-induced oxidative stress is also able to induce apoptosis, through three pathways: I) the ER-mediated pathway via ER stress and calcium release; II) the mitochondria-mediated pathway via direct and indirect activation of the mitochondria followed by caspase-dependent and/or -independent pathways; and III) the p53-dependent apoptotic pathway [56].

The Cd-induced production of ROS, TNF-α and NF-κB activates apoptosis through the Fas/FasL pathway [73]. This system is considered an important controller of renal cell apoptosis and can be induced also by different nephrotoxic agents [74]. When FasL is linked to Fas receptors, it causes apoptosis by inducing recruitment of the Fas associated protein and eventually leads to the activation of the caspases -3 and -9 [71, 75, 76].

In the kidney, a crucial role for the mitochondria dependent apoptosis is played by the Bcl-2 family proteins [77]; in particular, Bcl-2 protects cells from apoptosis, while Bax, a pro-apoptotic protein, induces programmed cell death. Exposure to Cd down-regulates Bcl-2 expression and up-regulates Bax in mice, thus confirming the role of this toxicant in the induction of apoptosis [78].

Recent studies [4] have demonstrated that, after Cd exposure, kidney tubular apoptosis is preceded by an induction of the autophagic processes, after which an up-regulation of Kim-1 expression and changes in the localization and function of N-cadherin [4] and claudin-2 [79], well known transmembrane adhesion molecules, are observed.

As Kim-1 is not detectable in normal kidney tissue and becomes evident in dedifferentiated epithelial cells of the proximal tubule after toxic injury, it might regulate cell-to-cell adhesion after Cd exposure. The appearance of Kim-1 is correlated to the loss of N-cadherin and claudin-2 which leads to changes in epithelial polarity and barrier function [80].

4. Cadmium toxicity in testis

Increased incidence of testicular cancer [81] poor semen quality and male infertility [82], delayed onset of puberty with impaired testicular growth [83], were observed in subjects exposed to Cd. However, differences in susceptibility between individuals living in the same or similar environments have been observed and referred to genetic polymorphism [81].

Testicular changes due to Cd toxicity have been observed in a variety of animal models at different stages of growth and maturity. Cd-induced testicular pathogenicity includes severe haemorrhage, edema, necrosis and atrophy, as well as reduction in counts and motility of sperm and decreased testosterone concentrations in plasma and testes [2].

Significant reduction in sperm concentration has been shown in rodents after Cd administration [84, 85], together with morphological abnormalities, reduced Johnsen's score [86] and increased death of sperm and Leydig cells [85]. Furthermore, desquamation of the germinal epithelium occurred in Cd-exposed rats [87], likely as a consequence of the loss of integral membrane proteins at the cell-cell interface of the blood testis barrier (BTB) and of germ cells adhesion in the seminiferous epithelium [88, 89].

Several studies have demonstrated that defective sperm formation, decreased sperm counts and consequent male infertility might be triggered by Cd owing to an increased lipid peroxidation with generation of ROS, such as superoxide anion radicals, hydroxyl radicals, nitric oxide and hydrogen peroxide [90], which are extremely toxic to spermatozoa, because of their high content of polyunsaturated fatty acids and their limited ability to repair DNA [91]. Oxidative stress can be evaluated through malondialdehyde (MDA) levels measurement [89]. In addition to the oxidative stress, a prominent role is played by inflammation mediated by TNF-α, which enhances and lengthens the inflammatory response by stimulating an increased expression of COX-2 and 5-lipoxygenase (LOX) and of their end-products PGE2 and leukotriene (LT)- B4 with further inflammation [20].

An important role in Cd-induced testis inflammation is also played by the cytokine Transforming Growth Factor (TGF)-β3, the most present form of TGF-β in testis, which is produced by Sertoli cells, spermatogonia, and early spermatocytes [92]. The effects of TGF-β3 on Sertoli cells tight junctions are mediated via p38 [93] and pERK1/2 [94] pathways. As a consequence of the TGF-β3 increase, a decreased content of occludin and ZO-1 in an *in vivo* model of Cd-induced infertility was observed [89, 94, 95].

Changes in the localization of cell adhesion proteins were observed either in cultured human Sertoli cells [97] or in animals exposed to Cd [90, 95]. In fact, Cd may cause severe damage to the testes inducing the disruption of the BTB [84] (Figure 1).

BTB is a testis-specific ultrastructural feature localized between adjacent Sertoli cells, controlling the movements of germ cells along the seminiferous epithelium [97]. Tight and adherens junctions are responsible for a selective permeability to nutrients, growth factors, ions and other small molecules and, in particular, for the progression of germ cells from the basal to the luminal compartment [98]. Therefore, the presence of occludin, claudin-11 and N-cadherin is requested for the maintenance of BTB integrity [95]. When testes are exposed to Cd, a reduction of the above mentioned proteins is observed. Consequently, the integrity of the BTB is lost [89, 98, 99].

Cd-induced oxidative stress and inflammation can also induce apoptosis *in vitro* [100] and *in vivo* [101]. In fact, Cd increases the expression of the proapoptotic Bax and of caspase-3 and reduces the expression of the antiapoptotic Bcl-xL [89, 101]. These changes induce the release of cytochrome C from mitochondria to cytoplasm and further activate caspase-3 activity, which finally results in cell apoptosis [101].

It was also observed that germ cell apoptosis might be the consequence of a Cd-induced reduction in testosterone level with detachment of germ cells from the seminiferous epithelium [102].

5. Therapeutic approaches in Cd toxicity

Numerous studies have been performed to find a therapeutic approach to neutralize, or at least reduce the toxic effects of Cd (Figure 1).

General agreement exists that there is currently no real clinical treatment for acute Cd intoxication [13]. Therapeutic approaches were based on the attempt to eliminate Cd with metal chelators, such as ethylenediamine-N,N,N',N'-tetraacetate (EDTA), dimercaptosuccinic acid (DMSA) and calcium disodium diethylenetriaminepentaacetate (DTPA) [103]. However, in particular for EDTA, an increased uptake of Cd by the kidneys and a higher risk of nephrotoxicity were observed [13].

For chronic Cd exposure, no chelation treatment has been recommended, even if there is considerable evidence of chelation's clinical efficacy of EDTA, DMPS, DMSA, and British Anti-Lewisite (BAL), able to increase urinary excretion of Cd. It has been suggested that the efficacy of EDTA is apparently improved with the associated use of glutathione, antioxidants including mannitol, thiamine, methionine, or zinc [104].

Particular attention has been paid to the possible prevention of Cd toxicity. In particular, the potential of many antioxidants, such as Coenzyme (Co) Q10, ß-carotene [105], and vitamin E [106], has been investigated, even if few showed positive results in clinical trials [107].

Therefore, a therapy able to counteract Cd toxicity is still lacking and the development of new therapeutic agents is requested. Natural products and dietary components have been investigated to find protective agents against the toxicity of alimentary and environmental Cd [58].

5.1 Therapeutic approaches in brain toxicity

During the last years many studies have been performed on the antioxidant properties of natural products, and in particular on their action on brain. Among them, *Physalis peruviana* belongs to the family of *Solanaceae* and showed protective effects against the Cd-induced oxidative stress [32]. Quercetin, the main polyphenol in *Physalis peruviana*, has various biological activities, such as antioxidant and anti-inflammatory functions. Moreover, *Physalis* extracts contain withanolides, natural steroidal lactones with anti-inflammatory activity [108]. In particular, quercetin was able to prevent the neurotoxic effect of Cd, owing to its radical scavenging and antiapoptotic activity [32, 109]. Therefore, *Physalis peruviana* could be considered a novel dietary therapeutic strategy against Cd neurotoxicity [32].

Similar antioxidant activity was demonstrated by the use of hesperetin and of hesperidin (hesperetin's 7-O-glycoside), flavonoids present in lemons and sweet oranges [110]. Flavonoids are lipophilic compounds, which cross the BBB, thus penetrating into the brain tissue. Hesperetin has a neuroprotective effect through the inhibition of oxidative damage, the restoration of altered mitochondrial respiratory enzymes [111] and the reduction of apoptosis in the brain of rats [110]. As to the hesperidin, it reduced Cd toxicity in the brain by different mechanisms, among which chelation of Cd, prevention of membranes damages, protection of thiol containing groups are included [40]. Therefore, both flavonoids possess the therapeutic potential to counteract the neurotoxic manifestations of Cd in the event of an exposure.

Melatonin, an effective antioxidant and free radical scavenger, showed efficacy in protecting Cd induced changes in the brain of rats through the control on the activity of acetylcholinesterase (AChE), lipid peroxidation and membrane bound ATPases [31].

Another compound with antioxidant effects is diallyl tetrasulfide (DTS), one of the major degradation products from garlic, present also as essential oil in *Adenocalymma alliaceae* and *Allium odorum L*. [112]. DTS reduces Cd induced biochemical alterations and decreases Cd in blood and tissues [112, 113]. *In vitro*, it lowers Cd induced generation of ROS, thus showing a protection from cellular damages [114]. As garlic promotes the survival of rat hippocampal neurons *in vitro* [115], DTS might produce positive effects on Cd induced lipid peroxidation in brain. In fact, lipophilic compounds such DTS easily cross the BBB and diffuse into the brain tissue [114], preserving the integrity of cellular membrane and normal physiological functions. An antiapoptotic effect of DTS was also shown when it was co-administered with Cd [30, 114].

A cytoprotective effect from Cd-induced neurotoxicity was also demonstrated for genistein. It is a plant derived phytoestrogen with antioxidant, antiapoptotic and anti-inflammatory effects. In Winstar rats [33], genistein induces its neuroprotective effects through the reduction in ROS generation and the inhibition of apoptotic signaling pathways, owing to its possibility to cross the BBB.

Among the compounds with possible therapeutic applications in the prevention of Cd-induced neurodegenerative changes, resveratrol has been also investigated [116]. Resveratrol (3,5,4′-trihydroxystilbene) is one of the most common and bioactive polyphenolic phytoalexins produced in plants, with promising potential against inflammation, both *in vitro* and in animal experimentation, dampening pro-inflammatory mediators and cellular pathways involved in

inflammation. Furthermore, resveratrol exerts neuroprotective effects on Cd induced apoptotic cell death and oxidative damage in neuronal cells [22].

5.2 Therapeutic approaches in kidney toxicity

Kidney, as above indicated, represents one of the main targets of Cd toxicity. Therefore, several therapeutic approaches were performed to prevent structural and functional damages following Cd environmental or experimental exposure. Recently, much attention has been focused on the protective functions of natural antioxidants against toxic heavy metals. Flavonoids are polyphenolic compounds widely distributed in dietary fruits, vegetables, and wine. In particular, quercetin is one of the most abundant, as it represents the 60-75% of the polyphenol ingestion. The administration of quercetin had a marked protective effect on Cd-induced nephrotoxicity even if administered after intoxication and might act through its antioxidant or anti-inflammatory or chelating properties [65].

As to the antioxidant effects, quercetin acts in a direct way by scavenging the superoxide anion and reducing the activity of superoxide-generating enzymes, such as xanthine and NADPH oxidase, and superoxide dismutase (SOD) [65], or in an indirect way through metallothionein MT-1 and MT-2 stimulation. The increased MT-1 and MT-2 expression after treatment with Cd plus quercetin protects against acute renal cadmium toxicity in rats, probably for its antioxidant properties [65]. As to the anti inflammatory effects, quercetin administration increases MT and endothelial nitric oxide synthase (eNOS) expression, and inhibits COX-2 and inducible nitric oxide synthase (iNOS) expression. In addition, quercetin has a potent chelating ability, thus decreasing Cd uptake and renal accumulation and suggesting a further protective effect against kidney toxicity [65].

Grape seed procyanidin extract (GSPE) is a natural compound found in fruits, vegetables, tea leaves and in the seeds of many plants, including grapes and apples. GSPE possesses several pharmacological and biochemical actions, in particular anti-inflammatory and antioxidant properties [117]. GSPE is a potent free radical scavenger more effective than vitamin E [118]. Recent studies have documented its efficacy in the prevention and attenuation of Cd-induced oxidative damage, as demonstrated by the amelioration of the increased levels of MDA and the increase of the glutathione peroxidase (GSH-Px) and SOD activities in the kidneys of the Cd plus GSPE mice [107]. Furthermore, GSPE could prevent Cd-induced renal apoptosis by interfering with the expression of the apoptosis related genes Bax and Bcl-2 [107].

As already demonstrated in brain, DTS has a protective effect in the tubular cells of kidney when challenged with $CdCl_2$ [55]. Its mechanism of action was related to antioxidant and metal chelating properties, with consequent cytoprotective functions.

Telmisartan, a selective angiotensin-1 receptor antagonist, was able to reduce the generation of ROS and the lipid peroxidation, and to decrease the overproduction of TNF-α, NO, NF-κB and iNOS in the kidney of mice challenged with Cd. Furthermore, telmisartan significantly lowered the Cd-induced expression of FasL and caspase-3 in kidney, so that an antiapoptotic activity related to its free radical scavenging and anti-inflammatory activity was proposed [71].

Among the naturally occurring antioxidants, betaine (glycine betaine or trimethylglycine) can be found in many foods, so that it is obtained from the diet or from its precursor choline [119]. In kidney, betaine plays a role in osmotic regulation, particularly in the urinary concentrating mechanism [120], and reduces oxidative stress [58]. In rats exposed to Cd, betaine decreased lipid peroxidation, enhanced antioxidants status and inhibited caspase-3 activity thus reducing tubular damage [58].

Taurine (2-aminoethanesulfonic acid), an essential amino acid, has been reported to protect against toxicity and oxidative stress following to heavy metals exposition [121, 122]. In Cd-intoxicated animals, pretreatment with taurine

prevented the structural damages and decreased the activities of the antioxidant enzymes [SOD, catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR), GPx and glucose-6-phosphate dehydrogenase] in the mice kidneys [123].

Similarly, naringenin (4,5,7-trihydroxy flavonone), a bioflavonoid found in citrus fruits, has nephroprotective [124] activities. In the kidney of Cd-challenged rats, naringenin significantly reduced the histological changes and reversed the activities of antioxidants and glutathione metabolizing enzymes [125].

Selenium (Se), an essential trace element, shows antioxidant activity by ROS scavenging [126], thus protecting the kidney from oxidative damage through the JNK phosphorylation activation [127]. During Cd exposition, one of the most important effects is depletion of Se in the body [128]. Its administration decreases ROS-mediated lipid peroxidation and regenerates glutathione [128], thus playing a cytoprotective role in the kidney. Furthermore, Se significantly inhibited the apoptosis due to ROS and mitochondrial dysfunction [34].

5.3 Therapeutic approaches in testis toxicity

It is well known that Cd may induce defective sperm formation, decreased sperm counts and consequent male infertility owing to an increased lipid peroxidation with generation of ROS [89]. Therefore, several antioxidant have been used to counteract Cd-induced impairment in spermatogenesis.

Among them, ascorbic acid was found to be essential for testicular differentiation, integrity and steroidogenic functions [90]; on these basis, ascorbic acid was shown to ameliorate oxidative stress during Cd exposure, enabling normal germ cell differentiation and protecting sperm count profile and morphology [129]. Furthermore, antioxidant therapy was useful in improving sperm quality in smokers [130]. In addition, the antioxidant activity of ascorbic acid was found to be therapeutically useful for the treatment of Cd induced germ cell apoptosis in testes [90]. Similar results were obtained in mice with the co-administration of vitamin E [90].

Alpha-lipoic acid is a natural antioxidant; in fact, exogenous supplementation with this substance increases free lipoic acid levels, so reducing oxidative stress both *in vitro* and *in vivo* [131]. Furthermore, lipoic acid possesses the ability to chelate heavy metals, such as Cd [102]. The concomitant administration of lipoic acid in rats subjected to Cd chronic toxicity provides protection against testicular damage induced by an increased induction of ROS, restoring the antioxidant status, normalizing steroidogenic events and reducing Cd accumulation in the testis [102].

Hesperetin occurs ubiquitously in citrus fruits as hesperidin, which is deglycosylated to hesperetin by intestinal bacteria prior to absorption [132]. Hesperetin exhibits various pharmacological activities, such as antioxidant, anti-inflammatory and metal chelating. *In vitro* studies indicate that hesperetin is a powerful radical scavenger [133]. In course of Cd intoxication, the supplementation with hesperetin significantly ameliorated the structural organization of seminiferous tubules, thus indicating a protecting role against Cd induced oxidative stress [134].

An important role in normal testicular development, spermatogenesis and spermatozoa motility is played by Se [135]. Improved sperm motility in subfertile men with low Se status and a protective effect against toxic metals on rodent testis were demonstrated after Se supplementation [135]. As to the mechanism involved, Se is an important component of selenoproteins, such as GSH-Px and thioredoxin reductases [136], which play a role in protecting cells against oxidative stress [137]. Se might also positively act on Cd-induced poor sperm quality and changes in spermatogenesis increasing testosterone levels by stimulating synthetic enzymes activity [138].

Another dietary antioxidant from food, able to attenuate the toxicity of environmental Cd on reproduction, is quercetin, a typical flavonoid found particularly in fruits and vegetables. The protective effect of quercetin on Cd-induced toxicity is based on the reduced generation of free radicals [65] with restoring GSH level and enzymatic antioxidants (SOD and

GSH-Px). Quercetin is also metal chelator [139] and prevents apoptosis by modulating the expression of Bax, Bcl-XL and caspase-3 in germ cells, thus resulting an important nutrient against the toxicity of environmental Cd on reproduction [140].

Melatonin, owing to its high lipophilicity, crosses biological membranes easily and is an effective anti-oxidant and free radical scavenger [141-142]. In addition, it has also been shown to have an anti-inflammatory effect and suppresses the synthesis of pro-inflammatory cytokines. Melatonin pretreatment significantly reduced Cd toxic effects by inhibiting MDA level, reestablishing GSH and SOD activities, and reducing TNF-α and IL-1β levels [143]. Therefore, melatonin shows a protective role against Cd-induced reproductive toxicity.

Coenzyme (Co) Q10, alone or in association with vitamin E, is very effective in the prevention of oxidative damage induced by Cd in rat testes [2]. CoQ10 is a co-factor in the respiratory chain, essential for the production of ATP [144]. It is a potent antioxidant interacting with other antioxidants such as tocopherol and ascorbate [144]. Pretreatment with CoQ10 and/or vitamin E prevented lipid peroxidation and protected the integrity and functioning of tissues and cells in the testes, inducing significative changes in enzymatic (SOD, CAT, GSH-Px, GR and GST) and non-enzymatic (Vitamins C and E) antioxidant systems [2].

Recently, the protective and therapeutic effects of resveratrol against $CdCl_2$-induced toxicity in rat testes were evaluated [145]. Resveratrol showed a protective action against Cd testicular toxicity as it upregulated Bcl2 and downregulated p53 and Bax gene expression. Furthermore, a marked improvement of sperm parameters and histopathological damages were observed in the resveratrol pretreated mice [146].

Among the therapeutic approaches to recover the toxic effects of Cd, flavonoids, used in conventional medicine for the treatment of different diseases, have been recently considered [89]. In particular, flavocoxid showed beneficial effects in protecting from Cd-induced damage of testicular functions, owing to its direct antioxidant activity, preventing the generation of MDA, to its anti-inflammatory activity, inhibiting COX-2 and 5-LOX , and reducing the synthesis of PGE_2 and LTB_4 [147]. Furthermore, flavocoxid showed an antiapoptotic activity, indicated by a significant reduction in Bax expression and an enhancement in Bcl-2 expression, and a counteracting effect on the hormonal unbalance induced by Cd [89]. Therefore, flavocoxid can be considered a strategic therapeutic agent to protect against male reproductive toxicity.

More recently, a protective effect of the adenosine A2A receptor agonist polydeoxyribonucleotide (PDRN) on Cd-induced changes was demonstrated [94]. PDRN is formed by a mixture of deoxyribonucleotide polymers of different lengths and nucleosides derived from salmon trout sperm [148]. In Cd challenged mice, PDRN administration reduced pERK 1/2 expression, modulated the hormonal status, ameliorated germinal epithelium changes and protected BTB ultrastructure, decreasing TGF-β3 immunoreactivity and enhancing its tight and adherens junctions. In particular, the reduction of TGF-β3, the most abundant form of TGF-β in testis produced by Sertoli cells, spermatogonia, and early spermatocytes [92], justified the improved localization of junctional proteins between Sertoli cells, thus demonstrating a protective effect of PDRN on Cd-induced damages of BTB [94].

6. Conclusions

The main challenges that remain with Cd can be related to the specific ability of this element to induce peculiar damages in various locations within the organism, resulting in acute and chronic poisoning. In particular, kidney, brain and testis are particularly involved in Cd noxious action, owing to its concentration within the nephron or to its capacity to overcome the BBB or the BTB. As a result, the glomeruli and the proximal tubules, the neurons and the seminiferous epithelium show morphological and functional changes, often irreversible.

As to the mechanisms responsible for Cd detrimental action, the main roles are played by oxidative stress, inflammation and apoptosis.

The therapeutic approaches described in the literature are mainly referred to the inhibition or downregulation of the oxidative stress in all the examined organs; nevertheless, it was observed that some drugs are able to modulate also inflammation and apoptosis, in all or in some considered organs with different, but often contemporary pathways involved.

However, a specific therapy able to counteract Cd toxicity is still lacking and the development of new therapeutic agents is requested.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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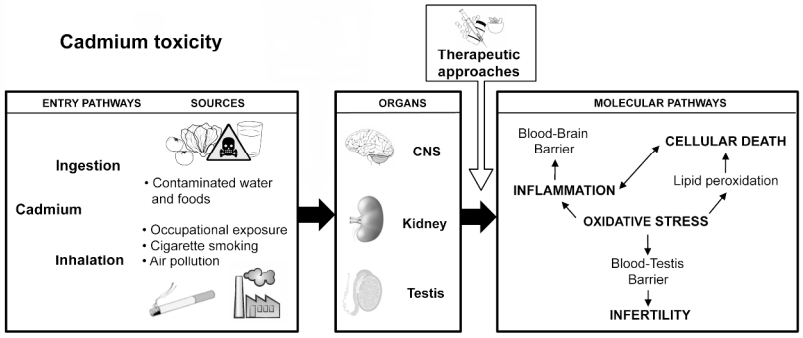
Figure legend

Figure 1 - The different entry pathways and sources of cadmium, the organs examined in the present review and the molecular pathways involved in cadmium toxicity are shown.



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