Biometals (2010) 23:811–822
DOI 10.1007/s10534-010-9314-4

Cadmium and cardiovascular diseases: cell biology, pathophysiology, and epidemiological relevance

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Received: 30 November 2009 / Accepted: 17 February 2010 / Published online: 7 March 2010

Abstract Today cardiovascular diseases (CVDs) are the killer number one world wide. In 2004 an estimated 17.1 million people died due to CVDs and this number will further increase to an estimated 23.6 million by 2030. Importantly, currently known risk factors, like hypertension, and hypercholesterolemia, can only be made responsible for about 50–75% of all CVDs, highlighting the urgent need to search for and define new CVD risk factors. Cadmium (Cd) was shown to have the potential to serve as one such novel risk factor, as it was demonstrated—in vitro, in animal studies, and in human studies—that Cd causes atherosclerosis (the basis of most CVDs). Herein, we discuss the molecular and cellular biological effects of Cd in the cardiovascular system; we present concepts on the pathophysiology of Cd-caused atherosclerosis, and provide data that indicate an epidemiological relevance of Cd as a risk factor for CVDs.

Keywords Cadmium · Endothelial · Smooth muscle cell · Necrosis · Apoptosis · Lipid · Cadherin · Divalent metal transporter 1 · Zinc · Inflammation · DNA damage

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Epidemiology

Even though CVDs are often regarded as being diseases of the developed countries, already today 82% of CVD deaths occur in low- and middle-income countries. Despite the fact that the dramatic number of deaths by CVDs could be rather easily prevented by life style modifications (quit smoking, healthy diet, and physical activity), this pandemic is far from being under control, and will increase dramatically, especially in the Eastern Mediterranean Region as well as in the Region of South-East Asia (WHO 2009). Based on the current status and the predicted developments, CVDs are and will remain the killer number one worldwide—at least for the next decades.

Pathophysiology

Atherosclerosis—the basis of most CVDs—starts early in life. The patho-biologically relevant changes in the vessel wall can be observed already in childhood and progresses slowly over decades, showing phases of activity, resting and even reversal of certain stages. For this reason, the dynamics including the underlying cellular and sub-cellular events are still far from being fully understood. Yet, the major steps in disease progression and important risk factors have been defined. Although there is some controversy regarding the initial event in the development of atherosclerosis, it is generally accepted that the vascular endothelium

plays a key role in disease initiation (Ross 1997; Hanke et al. 2001). The vascular endothelium plays physiologically a major role in regulating vessel tone, function, and homeostasis, anti-thrombosis, and is a selective barrier controlling compound and cell exchange between the blood stream and the vessel wall. In addition, the endothelium is a signalling platform between the vessel wall and the immune system. In disease initiation, changes in vascular endothelial function and/or morphology allow for an increased permeation of lipids through the endothelium and increased infiltration of the vessel wall by macrophages. Macrophages, due to an oxidation of LDL in the vessel wall, take up cholesterol via scavenger receptors in an uncontrolled way, leading to their trans-differentiation into foam cells. Deposits of cholesterol and foam cells in the sub-endothelial space, also called fatty streaks, are the first macroscopically visible changes in the vessel wall on the way from a healthy vessel wall to severe atherosclerosis (Crowther 2005). Due to a cytokine release from macrophages and foam cells, and due to the occurrence of necrotic cell death by cholesterol overload, the basis for an inflammatory state in the vessel wall is laid. It is an enduring inflammatory state which is thought to be the driving force underlying disease progression. Necrotic cell remnants stimulate cytokine secretion by various cell types leading to a chronic inflammatory state in the vessel wall and an attraction of various cells of the immune system as well as of smooth muscle cells (Ross and Glomset 1973; Crowther 2005). The tissue remodelling processes that are initiated by the above events include proliferation and cell migration-based thickening of intimal and medial layer of the vessel wall, and deposition of extracellular matrix. Further, due to hypoxia and cell death, free lipid pools are generated (Skalen et al. 2002). All these and other processes contribute to the formation of the atherosclerotic plaque. Plaques may cause a reduction in the vessel lumen and may—in an advanced state—be prone to rupture (Kadar and Glasz 2001). Ruptured plaques, which are essentially an "open wound" in the vasculature, cause the formation of thrombi. Loosening of thrombi may lead to embolism at downstream vessels and capillaries. The resulting hypoxia in tissues and organs downstream of the occlusion is the core element of severe clinical symptoms of CVDs like myocardial infarction, stroke, and peripheral arterial disease (Tedgui and Mallat 2001).

Risk factors

The spectrum of risk factors for CVDs ranges from purely genetic, to behavioural and environmental factors in the broadest sense. Apart from rather rare cases where CVDs are caused by a single risk factor (e.g. mutations in the ApoE gene), CVDs are initiated by a coincidence of different risk factors. The classical risk factors for CVDs include age, male gender, diabetes mellitus, hypertension, high blood cholesterol levels (LDL), and smoking. The latter four already show that behaviour and the environment (including the composition of nutrition) play an essential role in the majority of CVDs. When comparing individual patients, apparently CVDs develop at different sites in the vasculature. Patients differ in the time of onset, dynamics, and outcomes of CVDs, indicating the complex pathophysiology of CVDs. Different, genetically determined, susceptibilities to environmental risk factors, interactions of the cardiovascular system with organs like the lung, kidneys, and importantly also the immune system, as well as the mix of risk factors an individual is exposed to, are the likely causes of those differences (Ross 1995; Mallika et al. 2007).

Despite an increasing understanding of genes, proteins, signalling pathways, cell–cell interactions, and systemic processes involved in CVDs (initiation, progression, and outcome), the relevance of environmental factors is hardly investigated. However, findings like the correlation of chronic exposure to polluted air and CVDs, gives some hints that important environmental risk factors await discovery (Miller et al. 2007). Based on several publications from our group, in which we investigated the pathophysiology of smoking-caused atherosclerosis, we became interested in the relevance of metal ions in atherosclerosis initiation, as metals in cigarette smoke seem to constitute a highly relevant link between smoking and CVDs (Bernhard et al. 2005).

Cadmium in the vascular wall: entry, deposition, concentrations

In a small study comparing serum metal concentrations with the smoking status of healthy young volunteers, we found Cd to be significantly increased in smokers, with a tendency to be also increased in second-hand smokers. At that time, the correlation of

smoking and serum Cd concentrations was already well established in the literature, as smoking was already known to be one of the most important sources for Cd uptake by humans. In an important study by Abu-Hayyeh et al. the smoking status of humans was correlated with Cd concentrations in the three layers of the aortic wall (intima, media, adventitia). The finding of Cd concentrations in the media of up to 20 μM (compared to serum concentration of Cd in the low nanomolar range) showed clearly and for the first time that apart from liver, kidney and testis, also the vascular wall is a target organ of Cd deposition (Abu-Hayyeh et al. 2001; Prozialeck et al. 2006).

The precise uptake route of Cd by the cells of the vessel wall is unclear so far. Nevertheless, several ion channels and transporters have been described that are capable of transporting Cd across the plasma membrane. A number of those transporters are also expressed in endothelial cells. Importantly "the endothelial cell" is not a uniform cell type but endothelial cells differ, depending on e.g. the type of vessel (artery, vein, lymphatic system), vessel diameter, flow conditions, and importantly also the surrounding tissue (Garlanda and Dejana 1997). Since endothelial cells have to allow for an organ specific bidirectional transport of ions, molecules, and cells, specific differences between endothelial subgroups exist. Based on those differences—which are defined by specific changes in gene expression—endothelial subtypes not only differ in their uptake of Cd but it is also the endothelial subtype that defines the Cd selectivity of certain organs. Examples for endothelial organ specific channels and transporters for Cd uptake are ZIP transporters; e.g. ZIP8 that defines the specificity of Cd damage of testis and kidneys (He et al. 2009).

Another major, and yet not fully answered question is the form of Cd which is taken up by cells under physiological conditions i.e. the free or the bound ion or both. Given that the amount of free Cd in the circulation is very low compared to bound Cd, it seems likely that it is mainly the protein-bound Cd that enters cells. The group around Thevenod has addressed this issue in kidney cells and proposed that protein-bound Cd is taken up by endocytosis. Endosomal fusion with lysosomes and the low pH in endolysosomes leads to the release of Cd from proteins. Ionic Cd is finally transported into the cytosol via divalent metal transporter 1 (DMT-1) (Erfurt et al. 2003; Abouhamed et al. 2007; Wolff et al. 2008). Whether or not this mechanism is also active in endothelial cells, all components of this pathway are present and active in endothelial cells and might be a relevant route of Cd uptake by endothelial cells (Carraway et al. 2006; Gosselet et al. 2009). Importantly, it has to be mentioned that the situation (bound to free ion) in the circulation (the blood stream) need not necessarily reflect the situation in the microenvironment on the surface of endothelial cells. Other uptake routes for the free ion include ZIP8 (mainly responsible in testicular endothelial cell uptake of Cd), calcium-channels (Kyselovic et al. 2005), plasma membrane-associated DMT-1 (Simovich et al. 2002), and other ion pumps and transporters; the physiological relevance of these Cd uptake pathways in endothelial cell subtypes remains to be investigated (Prozialeck et al. 2008).

Apart from direct uptake of Cd by cells of the vessel wall, Cd may also be taken up by cells of the immune system, and may enter the vessel wall via infiltration of the vessel wall e.g. by Cd-laden monocytes (Steffensen et al. 1994). Since in the course of atherosclerosis, monocytes/macrophages trans-differentiate into foam cells, which—by cholesterol overload frequently die by necrosis—immune cell Cd pools may be released directly inside the vessel wall. In the above mentioned study by Abu-Hayyeh et al. the highest Cd concentrations in the vessel wall were found in the medial layer (Abu-Hayyeh et al. 2001). Based on this observation it would appear that endothelial cells allow for a sufficient transport of Cd across the endothelium, and also that the cells of the medial layer (mainly smooth muscle cells) are capable of retaining high amounts of Cd. However, another relevant route of Cd to the medial layer is based on the disruption of vascular endothelial cadherin-cadherin bonds (see Fig. 2b) and Cd-mediated endothelial cell death (see Fig. 2c), both leading to the formation of gaps between endothelial cells, allowing for a diffusion of Cd from the blood stream into the medial layer.

Cd-mediated signalling in cells of the vascular wall

Within cells, Cd—similar to circulating Cd—is mainly protein bound, and the major detoxification

system for Cd within cells is thought to be metallothionein-based, even though the existence of a direct protective effect of metallothioneins on Cd toxicity is somewhat unclear to date (Kaji et al. 1996; Kara et al. 2005; Fujiwara et al. 2009). The bioactivity of metallothionein-bound Cd is not well understood, but metallothioneins may—apart from binding and thereby inactivating the major portion of Cd ions—also serve as a source for constant levels of intracellular free Cd ions (in the sense of a chemical equilibrium) (Pearce et al. 2000; Prozialeck et al. 2006).

In the past years and decades several effects of intracellular Cd have been revealed. However, there is still uncertainty about the sequence of events, the separation of direct and indirect Cd effects as well as the identification of the major players and pathways that mediate Cd effects. This is particularly true for Cd effects on cells of the cardiovascular system.

We have previously performed microarray analyses of Cd-treated aortic endothelial cells which included an analysis of early responder genes after 6 h and secondary effects after 24 h of exposure (Bernhard et al. 2006). Not unexpectedly, a gene that was potently induced after 6 h was metallothionein 1 K (MT1K). After 24 h, five additional metallothioneins were upregulated. An upregulation of metallothioneins in response to Cd exposure of cells—also on the protein level—is well established in the literature and has also been observed in aortic endothelial cells (Kaji et al. 1993b). Among 11 genes found to be regulated after 6 h by low-dose Cd (1.5 μM) only 3 were upregulated (i.e. annexin A1 2.21-fold; MT1K 1.84-fold; and zinc finger protein 207 1.43-fold). Annexin A1 is supposed to be a cell migration repressor and zinc finger protein 207 expression was shown to correlate inversely with telomerase activity. All other genes, including vimentin and the proliferation repressor Kruppel-like factor 4 were downregulated. It is hardly possible to predict the exact cellular response to those gene regulations in detail, especially to predict the interactions of the gene products, a large number being transcription factors. However, what is clear from the data is that also low-dose Cd levels (well below toxic concentrations) alter endothelial gene expression and may thereby contribute to the initiation of pathophysiological changes in the vessel wall (Bernhard et al. 2006).

A well-known phenomenon associated with rather high—yet pathophysiologically relevant—concentrations of Cd is the disruption of endothelial integrity. Basically, there are two processes that cause this phenomenon. One is the disruption of vascular endothelial cadherin–cadherin bonds (associated with a reorganisation of the actin cytoskeleton) leading to a structural alteration of the vascular endothelium, which has been observed in numerous in vitro and animal experiments. This effect causes the opening of gaps between endothelial cells as well as the contraction of endothelial cells, which allows for increased permeability of the vascular endothelium (Sacerdote and Cavicchia 1983; Shukla et al. 1996; Prozialeck et al. 2006). The molecular details are still not clear yet, but the phenomenon is not specific for VE-cadherin and endothelial cells, is not associated with a loss of plasma membrane integrity, but seems to affect several members of the cadherin protein family and different cell types (Pearson et al. 2003; Woods et al. 2008). Since cadherin-cadherin bonds are Ca^{2+}-dependent, a direct interaction of Cd with Ca^{2+}-sites might account for this phenomenon. The second and well-described effect of Cd that alters endothelial integrity is the induction of endothelial cell death (see Fig. 1). Since endothelial cell death in vivo significantly alters the functionality of the vascular endothelium, the induction of endothelial cell death by Cd is thought to constitute a central phenomenon in the atherosclerosis promoting properties of Cd. Despite the fact that endothelial cell death by Cd has been observed in numerous studies, the data on the mode of cell death are contradictory. For example, Jung et al. described an induction of apoptosis by Cd in endothelial cells which was mediated by a p38 MAPK-dependent mechanism (Prozialeck et al. 2006; Jung et al. 2008). On the other hand, Dong et al. reported recently that low doses of Cd (below 10 μM) inhibit apoptosis induced by serum deprivation and basic fibroblast growth factor (bFGF), and that Cd induces autophagy (indicated e.g. by an upregulation of LC3; Dong et al. 2009). In a recent paper by our group (Messner et al. 2009) we found Cd to induce a programmed necrosis, or necroptosis in endothelial cell which relies on DNA-damage, activation of p53 (for background information on p53-mediated cell death see Moll et al. 2005), and signalling towards mitochondria (indicated by a potent inhibitory effect of over-expression of bclXL). Interestingly, caspase-3

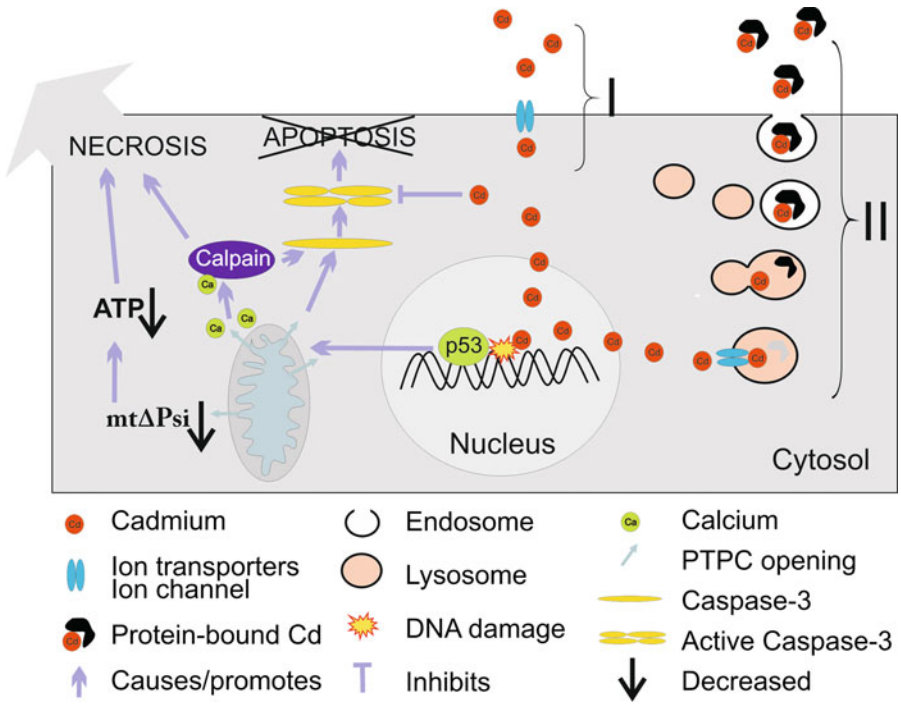

Fig. 1 Cd induces programmed necrosis. A hypothetical model for Cd-induced necrotic endothelial cell death induction. Cd is taken up by endothelial cells either directly via membrane associated ion transporters and ion channels e.g. divalent metal transporter 1 (DMT-1)…I, or in a protein-bound form via an endocytosis pathway…II. Free Cd ions diffuse into the nucleus and cause DNA damage—directly (e.g. by oxidative modification of bases) or indirectly (e.g. by inhibiting the DNA repair machinery)—which activates a DNA damage response. As a result of this p53-mediated response the endothelial cycle is halted (upregulation of p21/Waf1/Cip1 (not shown)), and a cell death signal is sent to the mitochondria (potential candidates are pro-apoptotic bcl2 family members). Due to opening of the permeability transition pore complex (PTPC), release and assembly of the apoptosome complex, caspase-9 is activated, which in turn activates caspase-3. By yet unknown processes Cd inhibits caspase activity and as an outcome of a decreased mitochondrial potential (mtΔPsi) due to opening of the PTPC, ATP generation is reduced, resulting in loss of function of ion pumps. The consequent influx of water finally leads to the rupture of organelles and the plasma membrane. Death execution is also supported by calpain which is activated by Ca^{2+} ions that are released from mitochondria by opening of the PTPC. The result is a necrotic phenotype of Cd-induced cell death

was found to be proteolytically processed, but caspase-3 activity was absent. A direct inhibitory activity of Cd on caspases has been described (Yuan et al. 2000). The outcome of the cell death observed in our study was necrosis (Messner et al. 2009). When comparing the modes of cell death by Cd that were described in other cell types, the situation is—compared to endothelial cells—even more complex. Although most concepts are not mutually exclusive, the reported pathways are highly diverse. These concepts include e.g. ER-stress mediated induction of cell death (Wang et al. 2009b), DNA-damage induced cell death (Liu and Jan 2000), Ca^{2+}-mediated cell death (Yang et al. 2007), ceramide- and calpain-mediated cell death (Lee and Thevenod 2008), mitochondrial depolarisation-dependent (Yang et al. 2007; Messner et al. 2009), JNK- and p53-dependent (Xu et al. 1999; Liu and Jan 2000; Messner et al. 2009), and reactive oxygen species-dependent (Yang et al. 2007) and -independent (Lag et al. 2002; Messner et al. 2009) pathways. Also in other cell types, apoptosis, necrosis, and autophagy in response to Cd exposure was observed (Xu et al. 1999; Yang et al. 2007; Wang et al. 2009a). Based on recent unpublished data from our group we think that in fact, most of the published mechanisms may be active but have not been properly linked to each other. Autophagy for example, does not per definition lead to the death of a cell. Hence, the activation of autophagy does not exclude apoptosis or necrosis to occur at the end of a cells life. Further, it cannot be excluded—and

is, in our view, rather suggested by the existing data—that several death pathways are active in parallel. Certain pathways may prevail in certain cell types (and may also differ among endothelial cell subtypes), whereas other observational differences may be based on cell type used i.e. primary cells or cell lines (which e.g. often lack p53). Another reason for contradictory explanations of the findings may purely be based on different methodological approaches. Independent of the form of cell death that Cd induces in endothelial cells (which might in vivo not only depend on experimental but also physiological conditions and concentrations of Cd), the death of endothelial cells will significantly hamper endothelial function by increasing endothelial permeability. Cd-induced endothelial necrosis would in addition to damaging the integrity of the vascular endothelium, also contribute to vascular inflammation.

Since cellular proliferation and angiogenesis play an important role in CVDs, the effects of Cd on those phenomena were also addressed in the past, yet to marginal extent. Cd was shown to inhibit both, endothelial proliferation (Messner et al. 2009) and angiogenesis (Woods et al. 2008). Inhibition of endothelial proliferation and angiogenesis significantly abrogates endothelial wound healing, another phenomenon that contributes to deterioration of endothelial function. Although the data on the signalling processes underlying Cd-caused inhibition of (endothelial) proliferation are generally sparse, based on our previous data we could suggest that Cd-caused cell cycle arrest is based on the DNA-damaging activity of Cd. As a result of DNA damage Cd leads to the accumulation of p53 (a marker for activation), and causes a cell cycle arrest via the subsequent upregulation of p53-downstream gene like p21/WAF1/Cip1, an inhibitor of cyclin-dependent kinases (Messner et al. 2009).

In the past many effects of Cd on cells have been ascribed to the oxidative stress promoting activity of Cd, which has also been observed in vivo (Sarkar et al. 1995). Despite a frequent description of oxidative stress after Cd exposure of various cell types, the situation in the vasculature is less clear. Oxidative stress induction i.e. the formation of H_2O_2, *OH, O_2* has been reported in endothelial cells in a few studies (Szuster-Ciesielska et al. 2000b; Martynowicz et al. 2004), whereas other studies failed to detect endothelial intracellular oxidative stress in response to Cd exposure (Lag et al. 2002; Messner et al. 2009). Importantly, Wolf et al. suggested that the concentration of Cd applied, as well as the upregulation of antioxidant defence in endothelial cells in response to Cd may define the presence of reactive oxygen species (ROS) in endothelial cells (Wolf and Baynes 2007). Based on these findings, the time of analysis and the dose of Cd applied might explain the different findings. Several reports showed that reactive nitrogen species, in particular nitric oxide, are reduced by Cd (Majumder et al. 2008; Yoopan et al. 2008).

A well known second messenger that may play an important role in Cd-mediated signalling is Ca^{2+}. In endothelial cells the occurrence and potential relevance of Ca^{2+} flux is not clear. An increase in cytosolic Ca^{2+} by Cd stimulation has the potential to open the mitochondrial permeability transition pore complex and to activate calpains. Both events have been reported to occur in Cd-induced cell death in other cell types (Yang et al. 2007; Lee and Thevenod 2008).

The effects of Cd on other vessel wall cell types have also been studied, though to a much lesser extent. Effects on smooth muscle cells include an interaction with ion homeostasis, and Ca^{2+} flux, cytotoxic effects, but also the stimulation of smooth muscle cell proliferation at low Cd concentrations, as well as an alteration of the smooth muscle cell cytoskeleton (Fujiwara et al. 1998; Kaji 2004; Prozialeck et al. 2008). The effects of Cd on vascular fibroblasts have hardly been studied so far. Of interest is a study reporting on an increased release of tissue plasminogen activator antigen from fibroblasts in response to Cd treatment. Via this mechanism Cd may alter the regulation of fibrinolysis (Yamamoto et al. 1996).

Pathophysiology of Cd-caused CVDs

As mentioned earlier, there are several opposing hypotheses to explain the initiation of cardiovascular diseases. Nevertheless, the mechanisms described may all be at play, and depending on the risk factor(s), different mechanisms may be dominant. Based on current knowledge, the hypothesis that best explains how Cd causes atherosclerosis is the "response to injury hypothesis" by Ross et al. In

this model it is a primary endothelial damage and/or functional impairment that initiates the diseases (see Fig. 2) (Ross 1999). Cd reduces endothelial barrier function by the disruption of endothelial cell–cell adhesions and by causing endothelial cell death, which has been demonstrated in vivo and in vitro (Sacerdote and Cavicchia 1983; Prozialeck et al. 2006; Messner et al. 2009). In conjunction with that, Cd-mediated inhibition of endothelial wound healing may cause a permanent shift from a closed towards a more open and permeable endothelium (Woods et al. 2008; Messner et al. 2009). Based on the "response to injury hypothesis", this damage allows for an increased and less controlled permeation and infiltration of the endothelium by cells of the immune system, and facilitates increased permeation and accumulation of lipids in the vessel wall. In fact animal studies provided evidence that lipids accumulate in several tissues including the heart and also the vascular wall in Cd-treated animals (Subramanyam et al. 1992; Messner et al. 2009). As another consequence of endothelial damage by Cd, extracellular matrix and cells below the endothelium i.e. smooth muscle cells and fibroblasts are exposed to factors present in the blood stream. A strong pro-thrombotic activity of extracellular matrix

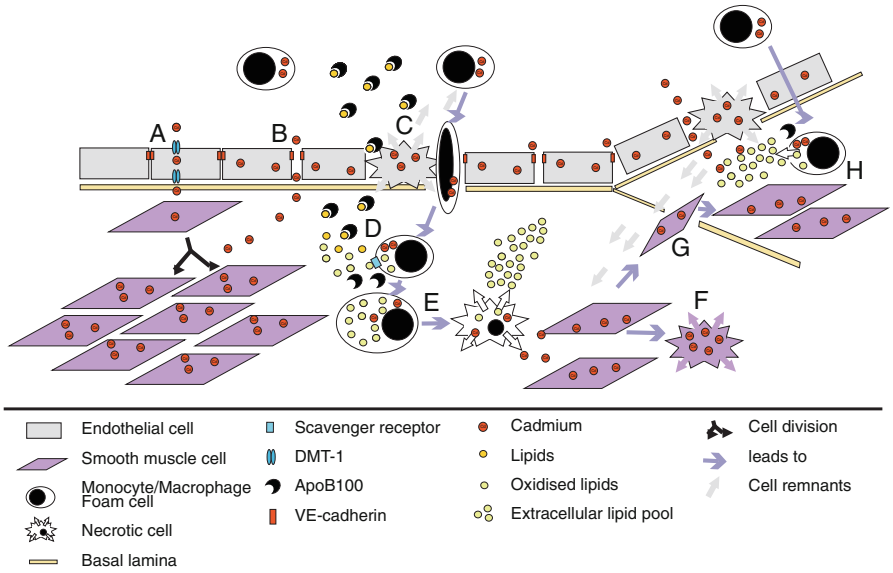

Fig. 2 Cd-induced pro-atherogenic changes in the vascular wall. A sketch of hypothetic events that Cd may induce in the vessel wall to initiate and promote atherosclerosis. **a** Apart from the uptake of Cd by endothelial cells, endothelial cells also allow for a transport of Cd to the subendothelial space. Cadmium is taken up by smooth muscle cells and initiates their proliferation, leading to thickening of the medial layer of the vessel wall. **b** Cd disrupts intercellular vascular endothelial cadherin–cadherin bonds, thus not only facilitating Cd diffusion into, but also enabling lipids and cells of the immune system to have access to the medial layer of the vessel wall. **c** Similarly, Cd induced endothelial cell death by necrosis also allows for increased access of lipids and cells of the immune system (e.g. monocytes) to the media. Furthermore, via the release of intracellular constituents by necrotic endothelial cells, cells of the immune system are attracted to the vessel wall, cause inflammation, and thereby contribute to **d** oxidative stress and lipid oxidation in the vessel wall. Oxidised lipids are taken up by macrophages via scavenger receptors in an uncontrolled fashion. Continuous uptake of lipids (cholesterol) by macrophages, which transdifferentiate into foam cells, start secreting proliferation-promoting and chemotactic cytokines as a response to stress. **e** By lipid overload foam cells ultimately undergo cell death by necrosis, which leads to the release of intracellular lipids, generating extracellular lipid pools. Extracellular lipid pools as well as necrotic foam cell remnants further promote an inflammatory state in the vessel wall. **f** Cd-mediated death of smooth muscle cells: these may also take up lipids, transdifferentiate into foam cells and contribute to free lipid pools and inflammation. **g** Activated by cytokines and attracted by cellular remnants and further cytokines, smooth muscle cells start to migrate into the subendothelial space, forming a neointima. **h** Accumulation of smooth muscle cells in the neointima, invasion of the neointima by macrophages, cell death of endothelial cells, smooth muscle cells, and foam cells, potently promote inflammation, initiating severe tissue remodelling processes including a deposition of extracellular matrix and free lipid pools. The process of atherosclerotic plaque formation and disease progression is initiated

components is well established and may contribute to thrombus formation. Furthermore, due to Cd-caused necrotic death of endothelial cells and other cells types in the vessel wall, cellular remnants are released. All these phenomena cause cellular stress resulting in secretion of cytokines. A vicious circle of damage, stress, and inflammation is initiated.

Other phenomena that have been observed in response to Cd exposure and initiate and promote atherosclerosis are hypertension (Tomera et al. 1994; Nakagawa and Nishijo 1996; Eum et al. 2008; Tellez-Plaza et al. 2008)—per se a highly important risk factor for atherosclerosis—and modifications of lipid profiles i.e. lipid oxidation (Sarkar et al. 1995), one of the prerequisites for foam cell formation and necrotic foam cell death; increased deposition of lipids in several organs (Subramanyam et al. 1992) including the vessel wall (Messner et al. 2009), and a modification of lipid profiles towards a more atherogenic state (a shift towards low density lipoprotein (LDL), very low density lipoprotein (VLDL) and a reduction in high density lipoprotein (HDL) particles) (Messner et al. 2009; Rogalska et al. 2009).

Nitric oxide (NO) is an essential factor in vasorelaxation, and decreased levels of NO have been shown to result in reduced vascular function (e.g. flow mediated dilatation) constituting a risk factors for atherosclerosis and CVDs. Cd has been shown to reduce NO levels (Majumder et al. 2008; Yoopan et al. 2008). Potential mechanisms include an inhibition of eNOS phosphorylation (Majumder et al. 2008), and an inhibition of eNOS expression by Cd (Yoopan et al. 2008).

As has been mentioned earlier, CVD progression and outcome highly rely on inflammation. Although Cd has clearly been linked to cellular damage, and inflammation of the lung (Kundu et al. 2009) and a Cd-caused systemic inflammatory status was observed (Mlynek and Skoczynska 2005), the interrelation of Cd (especially low concentrations) and vascular inflammation is unclear to date and hardly investigated. Vascular tissue remodelling processes, like plaque formation, induced by Cd represent per se an inflammation-associated phenomenon that indicates the presence of (at least) mild inflammation. Support for this hypothesis comes from in vitro analyses showing a release of cytokines like IL-6 (Messner et al., unpublished observation), and tumour necrosis factor alpha (TNFalpha) (only at low concentrations), as well as an expression of adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) by endothelial cells exposed to Cd (Szuster-Ciesielska et al. 2000a; Park et al. 2009). In support of this finding cadmium treated rats showed increases in liver cytosolic C-reactive protein (CRP) and serum levels (Agrawal and Bhattacharya 1989). On the other hand, no correlation between serum Cd levels and CRP levels in our study of humans could be observed (Messner et al. unpublished observations), suggesting that—within the limits of the study—increased serum Cd levels (that correlate with early vessel wall damage) do not cause an increase in markers of systemic inflammation in humans. Importantly, the population that was studied in the course of the mentioned project (Messner et al. 2009) was—in contrast to the study in rats (Agrawal and Bhattacharya 1989)—not exposed to high levels of Cd. In contrast to the above, several data support the hypothesis that Cd may also act anti-inflammatory e.g. microarray analyses of low-dose Cd effects on endothelial cells revealed a downregulation of cyclooxygenase 2 (COX2) and an upregulation of the cell migration inhibitor Annexin A1. (Bernhard et al. 2006) Other experiments revealed, that Cd inhibits vascular endothelial adhesion molecule expression in response to inflammatory stimuli, like lipopolysaccharides (LPS), viruses or TNFalpha (Szuster-Ciesielska et al. 2000a and Messner et al., unpublished observation). Due to the fact that Cd in vitro seems to possess both pro-, and anti-inflammatory properties, detailed histological analyses are needed to clarify this issue. It is suggested that the major factor that underlies the above "contradictory" findings is the dose of Cd applied.

Important aspects on the role of Cd in CVD progression e.g. plaque stability and vulnerability have not been investigated so far and need attention.

Cadmium and the heart

Starting with studies in the 1950s researchers also analysed effects of Cd on the heart. Basically the effects of Cd can be separated into two phenomena: (i) effects on tissue structure and integrity; (ii) effects on the cardiac conduction system. The former is reflected by sever damages to the heart muscle e.g.

necrosis and ultrastructural changes within cells (Kolakowski et al. 1983; Ozturk et al. 2009). The pathophysiology of these phenomena is not well established but a number of publications have suggested potential mechanisms; e.g. oxidative damage to the heart by an alteration of anti-oxidant defence and an increased generation of ROS (Jamall et al. 1989; Zikic et al. 1998; Wang et al. 2004), a reduction in coronary blood flow in isolated heart studies by Cd (Kisling et al. 1993), an inhibition of cardiomyocyte electron transfer chain by Cd (Wang et al. 2004), as well as a direct interactions of Cd with troponin C (Teleman et al. 1983) and myoglobin (Lepeshkevich and Dzhagarov 2009). Effects on the cardiac conduction system are based on interactions of Cd with contraction and pacing systems. Observed phenomena include, among others, a blockade of L-type calcium channel (Shen et al. 2000), and an alteration of outward potassium current (Follmer et al. 1992), both in ventricular myocytes; an interaction with calcium-activated and calcium-mediated processes in the heart (Prentice et al. 1984), and a blockade of fast Na channels of Purkinje fibres (DiFrancesco et al. 1985). In several of the above studies Cd effects on cardiac performance were described.

In vivo relevance and epidemiology

Probably the first study that indicated a cardiovascular activity of Cd in vivo was conducted by Alsberg and Schwartze in 1919, who observed a purple discolouration of rat testis after subcutaneous injection of Cd (Alsberg and Schwartze 1919). Since that time the relevance of Cd for CVDs has also been addressed in several epidemiological studies. Houtman et al. observed a higher than expected frequency of atherosclerosis in a Cd-contaminated area in the Netherlands (Houtman 1993). Other epidemiological studies on humans have revealed correlations of serum, blood, or urinary Cd levels to increased total mortality, hypertension, peripheral arterial disease, stroke, cardiac arrest, heart failure, and CVD mortality (Nakagawa and Nishijo 1996; Navas-Acien et al. 2005; Bhatnagar 2006; Tellez-Plaza et al. 2008). In a recent population-based study with 12,049 participants by Peters et al. increased blood and urine Cd levels were significantly associated with increased odds ratios (OR) for stroke and heart failure (Peters et al. 2010). Based on a small study of 197 healthy young adults (average age 20.6 years) we could recently show that increased serum Cd levels are also a risk factor for early atherosclerosis (increased intima-media thickness (IMT)) (Knoflach et al. 2009; Messner et al. 2009). Of potential therapeutic interest was that high Zn levels seemed to abrogate the effects caused by high Cd. When correcting for Zn levels, it could even be demonstrated that in the low Zn group and high Cd group the odds ratio for increased IMT was as high as 8. The relevance of these data is also supported by several in vitro studies indicating the protective effect of Zn (Kaji et al. 1992, 1993a; Messner et al. 2009) Despite some evidence for an epidemiological relevance of Cd in CVDs, it has to be clearly stated that—from the epidemiological point of view—the relevance of Cd as a risk factor for CVDs is still controversial (Navas-Acien et al. 2005; Plusquin et al. 2005; Schutte et al. 2008). Ideally, this issue should further be addressed by large cohort studies with follow-up and cardiovascular endpoints as readout, as well as prospective studies to evaluate the predictive potential of Cd levels in serum and urine for future cardiovascular events. Moreover due to the significant interaction of Zn with Cd effects observed in vitro and in animal studies in vivo, it is suggested to—in addition to analysing Cd levels in blood or urine—also analyse Zn levels, as it seems to be the amount of Cd PLUS the ratio between Cd and Zn that defines the bioactivity of Cd.

Summary

Cd is abundantly present in our environment. A potential pro-atherogenic effect—even if modest compared to other traditional risk factors—would have a significant impact population wide. Pathophysiologically relevant effects of Cd on the cardiovascular system have been defined, and could form the basis for medical interventions. The interaction of Zn with Cd effects may represent a starting point for such an intervention. Recent reports on the spreading of Cd on European agricultural land by using phosphate fertilisers shows that Cd contamination is still and will remain an important topic for our health (systems), and consequently also for research.

The understanding of the adverse health effects of Cd—particularly regarding CVDs—is, despite decades of Cd research, still at its start and several central questions remain to be answered; e.g. (i) the form, uptake, deposition, and bioactivity of Cd present in the circulation, in cells and cellular compartments; (ii) the search for Cd-sensitive proteins and receptors; (iii) the proper connection of observations to understand the Cd signalling network in different cell types; (iv) the concentration dependency of Cd effects; (v) the relevance of Cd in vascular inflammation; and (iv) the epidemiological relevance of Cd for CVDs.

Acknowledgement The authors would like to thank Mrs. Rajam Csordas-Iyer for correction of the English language. This project was supported by the Austrian National Bank (Project # 12697 to D.B.).

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