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SERUM VASOACTIVE AGENTS IN RATS POISONED WITH CADMIUM

HELENA MARTYNOWICZ, ANNA SKOCZYŃSKA, ANNA WOJAKOWSKA, and BARBARA TURCZYN

Department of Internal Medicine
Occupational Diseases and Hypertension
Wrocław Medical University, Poland

Abstract
Objectives: Mechanisms of the vascular effect of cadmium vary and involve nervous, hormone and intracellular signaling pathways. However, it is still not clear if mechanisms of the vascular effect of cadmium (Cd) include changes in the synthesis or release of vasoactive agents. The aim of this study was to evaluate the impact of subchronic Cd poisoning on blood nitric oxide or endothelin in blood and to relate it to the redox system activity in vessel walls and to blood Cd concentration. **Materials and Methods:** The study was performed on male Buffalo rats which were given cadmium in drinking water, 50 or 200 ppm, for 12 weeks. **Results:** The study showed different dose-dependent changes in toxicological and biochemical status. Mean serum nitric oxide concentration (measured using R&D Systems) was lower in rats poisoned with cadmium compared with the control group (57.7 ± 7.6 vs. control 65.0 ± 4.9 μmol/l, $p < 0.05$), whereas the plasma endothelin-1 level (measured using enzymoimmunoassay) and serum prostaglandin $PGF_{2\alpha}$ concentration (determined using R&D System) were similar in all animals. The lipid peroxides concentration (measured colorimetrically) was higher in the group treated with cadmium in a dose of 50 ppm than in controls (5.2 ± 3.0 vs. controls 1.4 ± 0.4 nmol/ml, $p < 0.001$) and gluthatione concentration was decreased in the group treated with cadmium in a dose of 200 ppm as compared with the control group, (1.3 ± 1.2 vs. control 2.5 ± 0.9, μmol/l $p < 0.05$). **Conclusions:** It is concluded, that cadmium induces oxidative stress in both doses, however, the activity of defending mechanisms depends on Cd dose. Oxidative stress can be responsible for decreased nitric oxide concentration in serum. We suppose that the mechanisms of the vascular effect of cadmium vary and are dose-dependent. Cd used in a dose of 50 ppm for three months induces more severe functional vascular disturbances than its dose of 200 ppm.

Key words:
Nitric oxide, Endothelin, Lipid peroxidation, Cadmium, Rats

INTRODUCTION

Cadmium (Cd) can disturb mechanisms of vascular tone regulation and induce hypertension in experimental models, and its effect is dose-dependent. Given in small doses, it induces hypertension due to vascular dysfunction. Used in large doses, it is responsible for renal tubules destruction and intraparenchymal fibrosis leading to nephrogenes hypertension. Mechanisms of Cd vascular effect vary and involve nervous, hormone and intracellular signaling pathways. Cadmium influences renin-angiotensin-aldosterone systems and atrial natriuretic peptide concentration [1–3]. It also affects calcium homeostasis by a possible increase in intracellular calcium ions concentration and evokes calcium mobilization [4–6].

However, it is still not clear, if mechanisms of the vascular effect of cadmium include changes in the synthesis or release of vasoactive agents such as nitric oxide (NO) or endothelin-1(ET-1). This question is essential in view of Cd dissemination in the environment and frequent occurrence of arterial hypertension and other cardiovascular diseases in the general population. Some epidemiological studies provide data indicating the role of cadmium in the development of spontaneous arterial hypertension [7–9], however, they often represent conflicting results [10–13].

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Address reprint requests to H. Martynowicz, MD, Department of Internal Medicine, Occupational Diseases and Hypertension, Wrocław Medical University, Pasteura 4, 50-367 Wrocław, Poland (e-mail: helenamar@poczta.onet.pl).

It is difficult to state conclusively whether the association between exposure to cadmium and the occurrence of the hypertension does really exist owing to the presence of some confounding factors, e.g., alcohol intake or tobacco smoking, which raise blood Cd levels.

In experimental studies performed on animals Cd enhances arterial blood pressure, depending on absorbed doses, time of exposure, route of Cd administration as well as on species and age of animals, and also their metabolic activity. It is supposed that the mechanism of pathogenic influence of cadmium on blood vessels is associated with the disturbance of vasoactive factors. The relationship between the occurrence of Cd-induced hypertension and changes in NO metabolism is confirmed by the results of some experiments performed on rats poisoned with cadmium.

In vitro experiments performed on isolated and perfused mesenteric superior artery of Cd-poisoned rats showed cadmium-induced changes in vascular reactivity to nitric oxide synthase inhibitor [14]. The results of numerous studies show that Cd-induced hypertension could result from the decreased synthesis and/or release and/or bioavailability of nitric oxide in vessel walls. On the other hand, in hypertensive Cd-poisoned rats a paradoxical growth of expression of NO synthase was observed [15,16]. Similarly, the results of measurements of endothelin in serum of Cd-poisoned rats and Cd-exposed humans were inconsistent; both the increased and the unchanged levels of endothelin-1 were shown [17–19].

Cadmium is known as a factor associated with oxidative stress. This metal is one of the agents, which can disturb balance between free radicals and antioxidants, leading to the increased superoxide anions concentration and vessel walls dysfunction. The increase in superoxide anions results in the decreased NO concentration and antioxidant enzymes activity (superoxide dismutase; SOD) in vessels of rats with hypertension [20]. Cadmium can also decrease antioxidants concentration [21], however, an increased concentration of antioxidants in experimental models has also been observed [22,23]. Such differences are probably associated with varied doses of the metal and exposure time. Cd given in low doses induces an adaptive/defence response, whereas large doses lead to the exhaustion of protective and adaptive mechanisms. On the other hand, the oxidative stress phenomenon associated with endothelium dysfunction participates in the development of hypertension and other cardiovascular diseases.

The aim of this study was to evaluate the impact of subchronic poisoning with cadmium on vasoactive agents such as nitric oxide, endothelin-1 and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in blood of rats. It is known that blood concentration of these agents represents the value resulting from the balance between their synthesis, release, distribution and elimination. However, the comparison of blood levels of the main vasoconstrictor and vasodilator (ET-1 vs. NO) could be useful in the estimation of vascular effect of cadmium.

The relationship between observed changes in the redox system activity in vessel walls should contribute to better understanding of the mechanisms by which Cd influences metabolism of vasoactive agents. Using cadmium in small hypertensive and large toxic doses we attempted to find out whether this influence depends on the degree of exposure to cadmium.

MATERIALS AND METHODS

The study protocol was performed according to the Polish law on animal protection [24]. The consent of Local Ethics Committee was obtained (No. 35/01). Thirty male Buffalo rats of body weight from 190 to 240 g were used in the experiment. The animals were maintained in a room with controlled humidity and temperature, and had free access to standard rat chow and water. The rats were given cadmium in drinking water, 50 or 200 ppm (cadmium chloride dissolved in distillate water), for 12 weeks (the rats were administered near 1 or 4 μg of cadmium daily). Control rats were given distillate water. All animals were observed for toxic symptoms of Cd effect and weighed once a week.

Rats were fasted starting the night before the experiment, and the next day were anesthetized intramuscularly with ketaminum in a dose of 300 mg/kg. The abdomen was opened through a midline incision, and the aorta was isolated. Blood obtained from the rat's heart was collected

into two samples in order to measure vasoactive agents and cadmium using EDTA-K_2 test-tubes (MedLab, no. 32.0301.2) or standardized glass-tubes. Atomic absorption spectrophotometer (SOLAAR M6, Thermo Elemental) was used to determine blood Cd. Cd levels were measured on graphite dish at wave length λ 228.8 nm, using background proof of Zeeman. The certificated standard of cadmium in the blood (liophilized) (Comunity Bureau of Reference, BCR) was utilized to prepare standard curve:

BCR 194: 0.2 ± 0.05 μg/l

BCR 195: 5.06 ± 0.15 μg/l

BCR 196: 12.33 ± 0.2 μg/l

The limit of detection was 0.05 μg/l of blood; linearity ranged from 0.01 to 12.33 μg/l of blood.

For the quantitative determination of serum total nitric oxide, NO_2^-/NO_3- assay of R&D Systems Europe (Abingdon, UK) was used. This assay involves the conversion of nitrate to nitrite by the enzyme nitrate reductase. The detection of nitrite was than determined as a colored azo-dye product of the Griess reaction that absorbed visible light at 540 nm. The concentration of NO was indirectly measured by determining both nitrate and nitrite levels in the sample. The relative levels of nitrate and nitrite can vary substantially, depending on ambient conditions and redox state of the given biological fluid. Therefore, the most accurate determination of total NO production requires quantitation of both nitrate and nitrite.

Plasma ET-1 was measured using BIOMEDICA, Wien enzymoimmunoassay. Prostaglandin $F_{2\alpha}$ in serum was determined using R&D System's $PGF_{2\alpha}$ Immunoassay (R&D Systems Europe; Abingdon, UK).

Five percent homogenates of aorta were prepared from thoracic fragment in saccharose buffer. Tissue samples were homogenized using homogenizer MPW 309 and centrifuged at 10 000 g for ten min at temperature 4°C. Determination of lipid peroxides (LPO) in homogenates was performed colorimetrically according to Satoh method [25]. We used thiobarbituric acid dissolved in sodium sulphate, and both liberation of lipid peroxides and color reaction were performed simultaneously by heating serum protein precipitate with this reagent in a weak acid solution. This method is specific and facilitates the precise measurements of serum lipid peroxide.

Glutathione (GSH) was measured using colorimetric assay BIOXYTECH GSH-400 (OXIS International, Portland, USA).

Statistical analysis

All values are expressed as means ± standard deviation. One-way analysis of variance for repeated measures was applied. The significance of differences between mean values was determined by *post hoc* comparison performed by Newman-Keuls test. A p value less than 0.05 was considered to indicate statistical significance. Each mean value was derived from ten rats. Statistical analysis was performed with STATISTICA 5.0 software.

RESULTS

Rats poisoned with cadmium in a dose of 50 ppm did not display any clinical signs of poisoning except slower putting on weight. Rats poisoned with cadmium in a dose of 200 ppm had yellow hair and teeth and they did not put on weight as quickly as those poisoned in a dose of 50 ppm (Table 1).

Cadmium present in drinking water in concentration of 50 or 200 ppm caused a significant increase in Cd concentration in blood of poisoned rats (Table 2).

The mean serum NO concentration was lower in rats poisoned with Cd in a dose of 50 ppm ($p < 0.05$) than in the control animals. The mean serum NO concentration in rats treated with cadmium in a dose of 200 ppm was also lower, but the difference was not statistically significant. The plasma ET-1 level and the mean serum $PGF_{2\alpha}$ level

Table 1. Body weight of rats poisoned with cadmium in a dose of 50, 200 ppm and controls during a 12-week period of poisoning. Each value represents the mean ± SD.

Time of the weight	Cd 50 ppm n =10	Cd 200 ppm n = 10	Controls n = 10
Start of the xperiment	198.9 ± 17.8	203.2 ± 10.9	203.8 ± 9.3
After 12 weeks	270.6 ± 33.9*	235.7 ± 19.7*	324.7 ± 24.8

* $p < 0.01$ vs. corresponding vehicle-treated group.

Table 2. Concentrations of serum nitric oxide, plasma endothelin-1, serum prostaglandin $F_{2\alpha}$ and blood cadmium in rats poisoned with cadmium in doses of 50 or 200 ppm and in controls. Each value represents the mean ± SD

Group	No. of rats	NO (μmol/l)	ET-1 (fmol/ml)	$PGF_{2\alpha}$ (pg/ml)	Cd (μg/L)
Cd 50 ppm	10	57.7 ± 7.6*	1.1 ± 0.2	5.5 ± 2.2	26.9 ± 3.4**
Cd 200 ppm	10	60.2 ± 5,7	2.2 ± 1.1	5.3 ± 2.3	67.3 ± 4.9**
Controls	10	65.0 ± 4.9	1.7 ± 0.6	6.1 ± 3.5	0.07 ± 0.01

* $p < 0.05$ vs. corresponding vehicle-treated group.
** $p < 0.001$ vs. corresponding vehicle-treated group.

Table 3. The concentration of lipid peroxides (LPO) glutathione (GSH) in aorta of rats poisoned with cadmium in doses of 50 or 200 ppm and in controls. Each value represents the mean ± SD

Group	No. of rats	LPO (nmol/ml)	GSH (μmol/l)
Cd 50 ppm	10	5.2 ± 3.0**	1.9 ± 0.7
Cd 200 ppm	10	2.2 ± 1.3	1.3 ± 1.2*
Controls	10	1.4 ± 0.4	2.5 ± 0.9

* $p < 0.05$.
** $p < 0.001$ vs. corresponding vehicle-treated group.

were similar in all groups of rats (Table 2). There was a negative linear correlation between serum NO and plasma ET-1 levels in the control group ($r = -0.74$, $p < 0.05$). Such a correlation was not observed in groups of rats poisoned with cadmium ($r = 0.09$, $p > 0.05$; $r = 0.16$, $p > 0.05$, in rats given cadmium in a dose of 50 and 200 ppm respectively) (Table 2).

Rats poisoned with Cd in a dose of 50 ppm displayed, in comparison with controls, the increased content of lipid peroxides ($p < 0.001$) in thoracic aorta. Homogenates of aorta obtained from rats poisoned with Cd in a dose of 200 ppm consisted a decreased amount of reduced glutathione ($p < 0.05$) (Table 3).

There was a positive linear correlation between lipid peroxides and glutathione levels in aorta of rats poisoned with Cd in a dose of 50 ppm ($r = 0.64$; $p < 0.05$) (Table 4).

DISCUSSION

The mean blood cadmium concentration in rats poisoned with Cd in a dose of 50 ppm (26.9 ± 3.4 g/l) was close to the

Table 4. Coefficients of linear regression between blood concentrations of cadmium (Cd, μg/L), serum concentrations of nitric oxide (NO, μmol/l), prostaglandin ($PGF_{2\alpha}$, pg/ml), plasma endothelin-1 (ET-1, fmol/ml), aorta glutathione (GSH, μmol/l) and lipid peroxides (LPO, nmol/ml) in rats treated with cadmium in a dose of 50 and 200 ppm and in controls (n = 10)

Group	ET-1	NO	$PGF_{2\alpha}$	Cd	LPO	GSH
Cd 50 ppm						
ET-1	1.00	0.09	-0.02	0.55	-0.12	0.23
NO	0.09	1.00	-0.05	-0.05	0.53	0.40
$PGF_{2\alpha}$	-0.02	-0.05	1.00	0.01	0.10	0.34
Cd	0.55	-0.05	0.01	1.00	0.17	0.40
LPO	-0.12	0.53	0.10	0.17	1.00	0.64*
GSH	0.23	0.40	0.34	0.40	0.64*	1.00
Cd 200 ppm						
ET-1	1.00	0.16	-0.19	-0.05	-0.64	0.53
NO	0.16	1.00	-0.69	0.06	-0.59	0.43
$PGF_{2\alpha}$	-0.19	-0.69	1.00	-0.35	0.57	-0.35
Cd	-0.05	0.06	-0.35	1.00	-0.56	-0.66
LPO	-0.64	-0.59	0.57	-0.56	1.00	-0.15
GSH	0.53	0.43	0.35	-0.66	-0.66	1.00
Controls						
ET-1	1.00	-0.74*	0.47	-0.06	0.03	-0.51
NO	-0.74*	1.00	0.15	0.01	-0.05	0.50
$PGF_{2\alpha}$	0.47	0.15	1.00	-0.33	0.23	0.15
Cd	-0.06	0.01	-0.33	1.00	-0.57	-0.01
LPO	0.03	-0.05	0.23	-0.57	1.00	-0.04
GSH	-0.51	0.50	0.15	-0.01	-0.04	1.00

* Statistical significance of coefficient r.
* $p < 0.05$.

value observed in the population occupationally exposed to this metal [26], whereas in rats poisoned with cadmium in a dose of 200 ppm (67.3 ± 4.9 μg/l) it was higher. It is known that subchronic poisoning with Cd given in concentration of 50 ppm in drinking water for three months induces persistent increase in arterial blood pressure in rats [27]. The present study shows that the impact of cadmium on vasoactive agents in blood is dose-dependent (50 or 200 ppm).

The endothelium is a target organ for cadmium toxicity. The decreased serum NO concentration was shown in *in vitro* experimental studies [28], whereas decreased NO bioavailability was observed in aorta of hypertensive rats [29]. The present data indicated different influence of cadmium

on serum NO concentration, depending on dose volume. Lower serum NO concentration can result from its worse production, augmented degradation and decreased functional pool of NO as compared with controls. Human essential hypertension and some animal models of hypertension are probably associated with the increased peripheral vascular resistance [30]. NO is known as a strong endogenous vasodilator, thus theoretical reasons for reduced NO production or bioavailability could lead to vasoconstriction and increased peripheral vascular resistance [31]. NO has been found to regulate the tone of normal vessels, including resistance vessels [32,33]. Nitric oxide can be changed by superoxide anion to form peroxynitrite ($ONOO^-$) reducing NO bioavailability [34]. Cadmium effect could be very harmful because it removes the beneficial, vasodilatating effects of NO, and increases constricting effects of $ONOO^-$. It is likely that the increased level of superoxide anions, associated with Cd-evoked oxidative stress, is one of the most important factors leading to hypertension in Cd-treated rats. The presence of "physiological" correlation between serum nitric oxide and plasma ET-1 concentration, shown in the present study only in the control group (Table 4), is typical of the group of rats with proper endothelium function. The absence of this correlation in the groups of rats treated with cadmium indicates Cd-induced disturbance of endothelial homeostatis.

The increased production of superoxide anions and so called oxidative stress in blood vessels is associated with cadmium toxicity. In the present study, it was confirmed by the increased lipid peroxides in aorta of rats poisoned with Cd in a dose of 50 ppm. The increased concentration of LPO in various organs was observed previously by other authors [35,36]. On the other hand, the gluthatione concentration was decreased in aorta of the rats treated with Cd in a dose of 200 ppm. Glutathione plays a defensive role against cadmium toxicity. The decreased glutathione level was observed in the organs of Cd-treated rats [21], however there are also contrary data [22,23]. These differences are probably associated with different doses of cadmium, and time of experiments. Glutathione concentration decreases in rats treated with cadmium in a dose of 200 ppm owing to the exhaustion of protective mechanisms. The observed unchanged glutathione concentration in rats treated with Cd in a dose of 50 ppm and decreased glutathione concentration in rats treated with Cd in a dose of 200 ppm, probably reflect the activity of adaptative and protective mechanisms. The present data confirm oxidative stress phenomenon. The enhanced nitric oxide biodegradation by superoxide anions in the vessel wall of Cd-treated rats could explain the decreased serum NO concentration. The observed positive linear correlation between LPO and GSH concentrations in the group of rats poisoned with Cd in a dose of 200 ppm indicates the onset of defensive mechanisms activity in vessel walls. The absence of such a correlation in the group of rats treated with cadmium in a dose of 200 ppm indicates the exhaustion of protective mechanisms. In addition, this study revealed that cadmium given in a dose of 50 ppm showed a stronger pro-oxidative activity, inducing higher LPO concentration in aorta and lower serum NO concentration than Cd given in a large dose of 200 ppm.

To sum up, this study shows different, dose-dependent changes in the toxicological and biochemical status of vasoactive agents concentration in serum of Cd-poisoned rats. Cadmium used in a dose of 50 ppm for three months can induce more severe functional vascular disturbances than Cd used in a dose of 200 ppm. Probably cadmium used in large doses, including a dose of 200 ppm for three months, results more in toxic effects, than in vessels dysfunction.

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