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Cardiac Tissue Injury Resistance During Myocardial Infarction at Adulthood by Developmental Exposure to Cadmium

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Abstract It has been suggested that prenatal exposure to cadmium may alter the cardiovascular function during adulthood. Using the left coronary artery ligation model of acute myocardial infarction, we studied the cardiac function of female adult offspring rats exposed to cadmium (30 ppm) during gestation. The cardiac ischemic zone in the control and cadmium-exposed groups was measured 72 h post-ligation using the TPT staining technique. Offspring from cadmium-treated dams showed a significantly smaller infarcted area compared with the control group (7.1 ± 1.5 vs. $19.6 \pm 2.8\%$, $P \leq 0.05$). We also performed echocardiographic and biochemical studies, which positively correlated with the differences observed previously. To evaluate whether the effects were associated to pre-infarct tissue damage and/or angiogenic molecules, we performed histological studies and measured the expression of vascular endothelial growth factor (VEGF), and platelet endothelial cellular adhesion molecule-1 (PECAM-1). Results revealed a higher heart vascularization in the exposed offspring that was associated with an increase in PECAM and a decrease in VEGF expression. We conclude that prenatal exposure to cadmium induces fetal adaptive responses involving changes in the expression of some cardiac angiogenic molecules resulting in long-term resistance to infarction.

Keywords Cadmium · Infarction induction · Echocardiography · VEGF · PECAM

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Introduction

Adverse environmental conditions during intrauterine life may be predictive of several pathologies in adult life including cardiovascular disease (CVD) [1]. It has been demonstrated that direct exposure to cadmium (Cd^{2+}), a ubiquitously distributed environmental toxicant, and main component of tobacco smoke, may be involved in CVD including hypertension, atherosclerosis, and myocardial infarction, probably due to endothelial dysfunction [2–6]. Cardiac hypertrophy and endothelial dysfunction may occur as adaptive responses to an increase in the heart workload to maintain cardiac function. However, if these adaptive events persist for longer periods of time, it can cause cardiac dysfunction leading to heart failure [7]. We have recently reported that exposure of pregnant rats to 30 ppm of Cd^{2+} induces a reduction in the endothelium–dependent activity and a concentric ventricular hypertrophy in the adult offspring, even if Cd^{2+} was not detected in the analyzed tissues at various age periods [8]. These results, together with increased NF-*k*B expression in placental tissues, suggest that molecular events induced by Cd^{2+} such as oxidative stress may occur in placentas exposed to Cd^{2+}, thus affecting long-term cardiovascular reactivity of the offspring [8]. These mechanisms may involve changes in the expression of specific genes that affect the phenotype of fetal cardiac physiology.

Myocardial infarction (MI) is a common CVD that represent one of the leading causes of death in many countries throughout the world. After MI, restoration of cardiac function requires increase of the myofibroblast population, but also a re-vascularization of the injured region [9]. In addition, promoting early re-opening of the damaged vessels in the injured heart after MI can improve cardiac function and reduce mortality [9]. MI is associated

with an inflammation reaction and with release of angiogenic factors, which may be critical for new vessel formation in the healing area contributing to cardiac repair [10–12].

Formation of new blood vessels is critical for supplying the healing infarcted myocardium with oxygen and nutrients necessary to sustain metabolism. Angiogenesis is dependent on a complex interaction between extracellular matrix, endothelial cells and pericytes in response to an imbalance in the presence of angiogenic compared with angiostatic factors in the local environment. The platelet/endothelial cell adhesion molecule 1 (PECAM/CD31) and the vascular endothelial growth factor (VEGF) are angiogenic factors produced by a variety of cells, including endothelial cells, with specific receptors on the endothelium [13]. Both molecules are important mediators of neovascularization in physiological and pathological conditions, playing crucial roles in the developmental blood vessel formation and regulation of hypoxia-induced tissue angiogenesis [14].

In this work, we explored whether the effects induced by developmental Cd^{2+} exposure affect cardiac response to injury such as acute ischemia. Using a rat model of myocardial infarction by ligature of the left coronary artery, we evaluated the cardiac function of adult offspring exposed to Cd^{2+} (30 ppm) during gestation and controls 72 h after ligature. To assess whether the prenatal Cd^{2+} treatment caused programming of the CV function, we determined the cardiac expression of VEGF and PECAM/CD31 before MI, and the magnitude of the cardiac tissue damage after MI.

Methods

Animals and Treatments

Virgin female Wistar rats (60 days, 200–250 g weight) were caged with mature male breeders, and mating was confirmed by the presence of a vaginal copulation plug (day = 0 of gestation). Upon confirmation of mating by the appearance of a semen plug on the cage floor, the rats were randomly allocated into control group (distilled drinking water, $n = 8$) or Cd^{2+} treated group by addition of $CdCl_2$ in the drinking water (30 mg/l, 30 ppm of Cd^{2+}, $n = 8$). Females were fed ad libitum from conception to spontaneous delivery (on day 21 approximately) with a standard isoenergetic diet for rats (Champion, Co., Santiago, Chile) composed by 12.7% protein, 33.4% total fat, and 522 kcal per 100 g of food. Litters were reduced to 8 (approximately equal males and females) within 24 h of delivery and continued with breastfeeding until weaning (22 days old). Because of some reports indicating higher susceptibility of females than males to toxic metals exposure (including Cd^{2+}) [15], our experiments were conducted only in female offspring. After weaning, two females per group ($n = 16$) were randomly chosen and separated in cages with four rats each, and were fed with the isoenergetic diet and distilled drinking water until 60–70 days. One female per group ($n = 8$) was killed after 6 h fasting; the hearts were removed, halved, and set aside for histological and WB analysis. Echocardiographic analysis was performed before and after the MI induction on the remaining offspring ($n = 8$ per group). Seventy-two hours post-infarction, the heart ischemic area was measured, blood was collected in EDTA, plasma separated and frozen at −80°C for biochemical measurements.

Induction of Acute Myocardial Infarction

Rats were anesthetized with a mixture of Ketamine/HCl and Xylazine (100 mg/5 mg/kg, i.p.), and the left chest was opened through the fourth intercostal space. The pericardium was opened, and a 5.0 prolene suture was tightened around the proximal left anterior descending (LAD) coronary artery (approximately 2 mm from its origin and before the first branch of diagonal artery) as described elsewhere [16]. In sham-operated animals, the same procedure was performed and the suture was left in place loosely. The heart was immediately internalized, the chest was closed, and negative pressure artificially restored to reverse the procedure-induced pneumothorax. The muscle layer and the skin were sutured separately, and the animals were allowed to recover.

Infarct Size Assessment

Three days after myocardial infarction, infarct size and area at risk were measured with the triphenyltetrazolium (TPT) staining technique [17]. The hearts were cut into five to six slices (2 mm thick) parallel to the atrioventricular groove, and weighted. The slices were incubated in a 1% solution of TPT for 30 min at 37°C and then kept in 10% formaldehyde solution for 24 h. The viable tissue is stained red with TPT, while the dead tissue (infarcted tissue) remains unstained. The volume of the infarcted and non-infarcted zones were measured by planimetry of the corresponding area on the cross-sectional surface of each slice, and then multiplied by the thickness of the slice. These volumes were added across the slices to obtain the corresponding total volumes of the two regions. The infarct size was calculated in both groups ($n = 8$ per group) as a percentage volume of the infarcted area (white area) versus the risk area (non-blue area).

Echocardiographic Analysis

Echocardiographic studies were performed before and after 2 days of experimental MI. Offspring (60–70 days old) of control and Cd^{2+}-treated rats were anesthetized with Ketamine/Xylazine (40 mg/3 mg/kg, i.p.). Images were obtained using Sonosite 180 plus echocardiograph equipped with an electronic 10-MHz linear-array transducer. The following parameters were measured: aortic diameter (AD), left ventricular end-systolic cavity (LVESC), LV end-diastolic cavity (LVEDC), LV ejection volume (LVEV), and LV fractional shortening % (FS). FS was calculated according to the formula FS = [(LVEDV − LVESV)/LVEDV] × 100(%) [14].

Determination of VEGF and PECAM by Western Blot

Frozen hearts from offspring adult rats, control and exposed to Cd^{2+}-during gestation, were thawed and homogenized using ice-cold radio-immuno precipitation assay buffer (RIPA)(Thermo Scientific, Rockford, USA). Homogenates were centrifuged at 12,000×*g* for 10 min to remove nuclei and cellular debris, and supernatants were used for Western blot analysis. Total protein content was determined by using a commercial assay kit (Bio-Rad, Hercules, CA, USA). All steps were carried out at 4°C unless stated otherwise. Aliquots of protein lysates (50 μg) were separated using 10% polyacrylamide gel (PAGE) containing sodium dodecyl sulfate (SDS) along with a pre-stained, broad-range, molecular weight marker (New England Biolabs Inc., MA, USA). The separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Thermo Scientific, Rockford, USA) overnight at 4°C. The membranes were then blocked with 5% non-fat milk in Tris-buffered saline with 1% Tween-20 (TBS-T) for 1 h, and then incubated with specific primary antibodies against PECAM (Chemicon, MA, USA) at a dilution of 1:5,000 or VEGF (Abcam, Cambridge, UK) at dilution 1:10,000 in buffer TBS-T containing 5% non-fat milk overnight at 4°C. For internal control, membranes were incubated with primary antibody against *β*-actin (Abcam, Cambridge, UK) at a dilution of 1:7,000. The membranes were washed 4 times with TBS-T for 5 min each and incubated for 1 h with goat anti-mouse secondary antibody–horseradish peroxidase conjugates (Chemicon, MA, USA) at a 1:1,500 dilution. After the membranes had been washed thoroughly, specific bands were developed using an enhanced chemiluminescence (ECL) Western blotting detection kit (Lightning Plus-ECL, Perkin Elmer, MA, USA) and exposing the membranes to film (Amersham hyperfilm TM ECL, Gen. Electric Health Care Limited, Buckinghamshire, UK) to obtain a signal. Bands were measured using the densitometry tool of Image J software.

Histological Examination

Hearts from adult female offspring from control and Cd^{2+}-treated groups were fixed with bouin and embedded in paraffin. Four-micrometer sections were stained with haematoxylin-eosin for morphological analyses [18].

Statistical Analysis

For animal studies, data were expressed as mean ± SEM for indicated number of animals. To define normality within all variables, Shapiro–Wilk test was used. For variables without normal distribution, the nonparametric Kruskal–Wallis (among groups) and Mann–Whitney *U* test (between two groups) were applied to assess statistical differences. Results were considered significant at $P < 0.05$.

Results

Infarct Size

The ischemic zone in the hearts of Cd^{2+}-treated offspring was significantly lower than the control animals (Fig. 1). The magnitude of the ischemic zone was 19.6 ± 7 and 7.1 ± 1.5% (mean ± SEM, $n = 8$, $P \leq 0.05$) for hearts in control and Cd^{2+} treated animals, respectively.

Echocardiographic Studies

Echocardiographic analyses were performed before and 2 days after the ischemia induction in order to check the cardiac function (Fig. 2). In the pre-infarction condition, the exposed offspring presented a significant decrease in the ventricular cavity during diastole that was associated with a significant thickening of the anterior wall during both diastole and systole. This finding confirmed a previous report recently published by our laboratory [8]. Control animals presented a significant commitment of the cardiac function with morphological and functional alterations as a consequence of the ischemic damage induced by the experimental MI. Echocardiography shows that the left ventricular cavity was dilated due to a significant increase in its diameter during both systole and diastole (LVEDC and LVESC, Table 1) associated with a significant thinning of the anterior and posterior wall thickness (ALVSWT, ALVDWT, PLVSWT and PLVDWT, Table 1). Furthermore, we observed a decrease in the contractility function evidenced by a significant decrease in fractional shortening, an indicative of systolic failure (FS%, Table 1). On the contrary, offspring exposed to Cd^{2+} during development

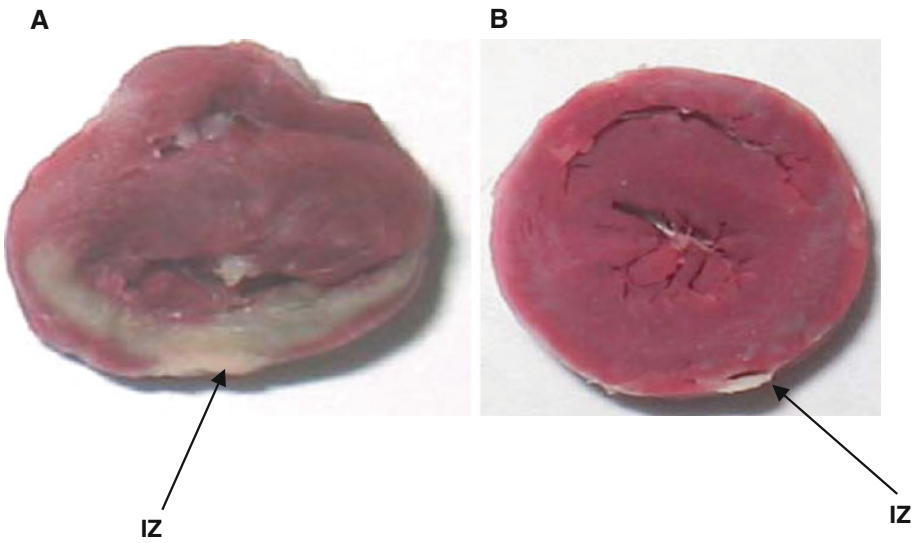

Fig. 1 Hearts showing the ischemic zone (*IZ*, *arrows*) of the control (**a**) and Cd^{2+}-treated rats (**b**) after 72 h of a ligation of the left anterior descending coronary artery (*LAD*). Evaluation of the size of the damaged zones was assessed by planimetry as described in "Methods" ($n = 8$)

showed a protective effect to MI induction, as shown in the echocardiographic parameters (Table 1; Fig. 2).

Expression of PECAM and VEGF in the Heart of Control and Cd^{2+}-Treated Offspring

We studied whether heart tissue of control and Cd^{2+}-treated offspring before MI expressed some angiogenic molecules in a way that could explain, at least in part, the resistance to MI observed in treated animals. We determined PECAM/CD31 expression in hearts of both groups by Western blotting (Fig. 3). Results indicated increased levels of the PECAM/CD31 expression in the Cd^{2+}-treated offspring compared with control.

Because angiogenesis could be also related to VEGF, we measured its expression in hearts of control and Cd^{2+}-treated offspring. Results showed decreased levels of VEGF protein concentration in the heart of the Cd^{2+}-treated offspring (Fig. 4).

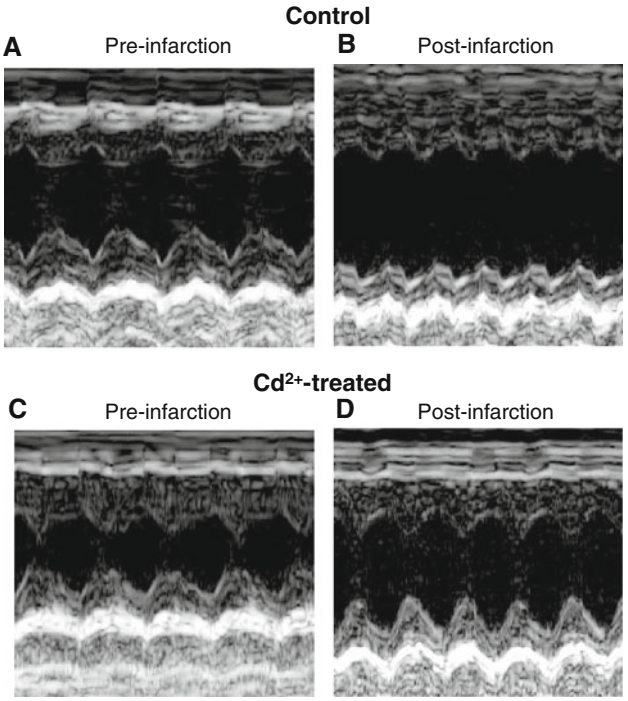

Fig. 2 Echocardiography of left ventricular-end diastolic cavity (*LVEDC*) of control animals before (**a**) and post-ligation of proximal left anterior descending coronary artery (*LAD*) (**b**). **c**, **d** Show the echocardiographic images of the adult Cd^{2+}-treated offspring before (**c**) and after the coronary artery ligation (**d**)

Histological Analysis in Hearts of Control and Cd^{2+}-Treated Offspring

Histological analysis obtained from hearts of both groups indicates the existence of morphological changes in the Cd^{2+}-treated group compared with control offspring. Arrows indicate the number of cord-like structures in the heart, which are more abundant in the treated offspring compared with control (Fig. 5).

Biochemical Measurements

To evaluate the content of different plasma markers of health, we made a complete plasma analysis in both control and Cd^{2+}-treated groups 72 h post-infarction. When comparing both groups after MI, no biochemical parameter was altered but lactic dehydrogenase (LDH) and glutamic oxalacetic transaminase (GOT). These enzymes may increase after tissue damage, especially after cardiac tissue damage [19]. The higher post-infarction levels of LDH and GOT in the control group suggest and increased cardiac tissue damage after MI, which was not observed in the Cd^{2+}-treated group.

Table 1 Echocardiographic parameters in adult offspring of control and Cd^{2+}-treated adult rats before and 3 days after ligation of coronary artery

	Control		Cd^{2+}-treated	
	Pre-infarction	Post-infarction	Pre-infarction	Post-infarction
LVEDC (mm)	6.0 ± 0.05	7.0 ± 0.04*	5.0 ± 0.04$^{\Upsilon}$	6.0 ± 0.05*
LVESC (mm)	3.0 ± 0.04	5.0 ± 0.04*	2.8 ± 0.05	2.9 ± 0.04
ALVSWT (mm)	2.4 ± 0.2	1.7 ± 0.2*	3.5 ± 0.3$^{\Upsilon}$	2.4 ± 0.2*
ALVDWT (mm)	1.5 ± 0.1	1.2 ± 0.1*	2 ± 0.2$^{\Upsilon}$	1.7 ± 0.2
PLVSWT (mm)	2.7 ± 0.3	1.8 ± 0.1*	2.7 ± 0.2	2.7 ± 0.1
PLVDWT (mm)	1.8 ± 0.2	1.3 ± 0.04*	1.9 ± 0.1	1.8 ± 0.2
AD (mm)	0.2 ± 0.02	0.2 ± 0.04	0.2 ± 0.02	0.2 ± 0.02
LVEV (ml/beat)	0.2 ± 0.08	0.21 ± 0.08	0.21 ± 0.03	0.19 ± 0.05
LVFS (%)	49 ± 2	32 ± 2.5*	52 ± 7	51 ± 7

LVESC left ventricular end-systolic cavity, *LVEDC* left ventricular end-diastolic cavity, *ALVSWT* anterior left ventricular-diastolic wall thickness, *ALVDWT* anterior left ventricular-systolic wall thickness, *PLVSWT* posterior left ventricular-systolic wall thickness, *PLVDWT* posterior left ventricular-diastolic wall thickness, *AD* aortic diameter, *LVEV* left ventricular ejection volume, *LVFS* left ventricular fractional shortening. Values are mean ± SEM, $n = 8$; * $P < 0.05$, compared with pre-infarction condition within each experimental group. $^{\Upsilon}$ $P < 0.05$, compared with control pre-infarction condition. Kruskal–Wallis and Mann–Whitney *U* test

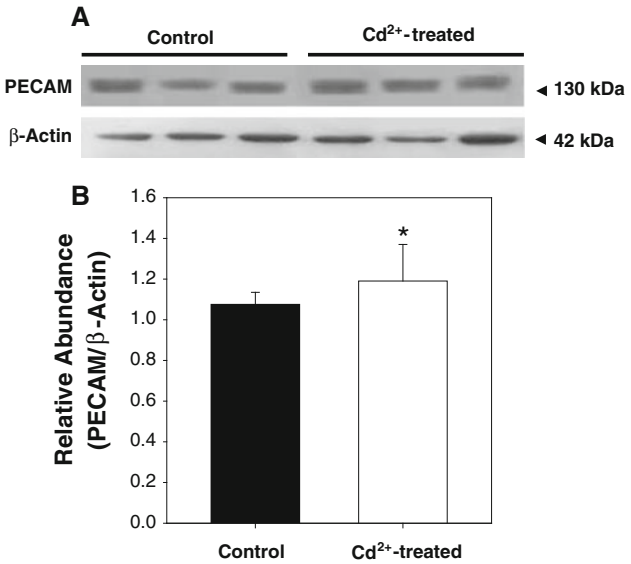

Fig. 3 WB of PECAM in hearts of control and Cd^{2+}-treated female rats related to β-actin (**a**). Quantification of bands by densitometry (**b**) is expressed as relative abundance of the ratio PECAM/β-actin; $n = 6$, * $P \leq 0.05$

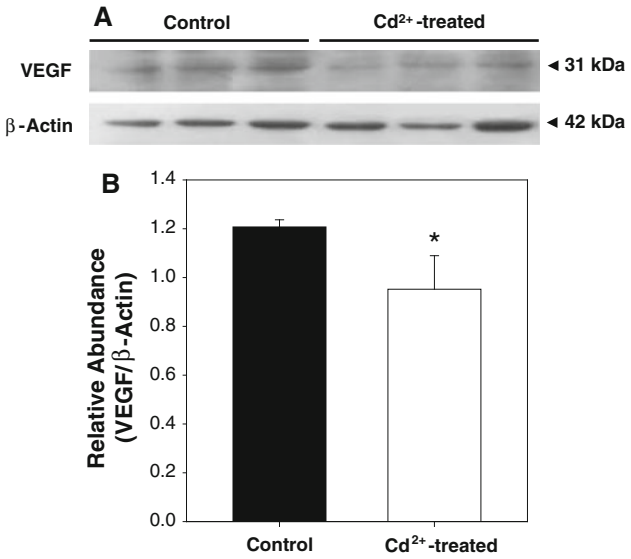

Fig. 4 WB of VEGF in hearts of control and Cd^{2+}-treated female rats related to β-actin (**a**). Quantification of bands by densitometry (**b**) is expressed as relative abundance of the ratio VEGF/β-actin; $n = 6$, *$P \leq 0.05$

Discussion

In this study, we have demonstrated that maternal exposure to Cd^{2+} during pregnancy in rats may induce long-term adaptive changes in the cardiovascular function of the offspring by enhancing the resistance against ischemia-induced cardiac injury during myocardial infarction. We hypothesized that this adaptive response may have its origin in the morphological and functional alterations induced by Cd^{2+} during development. Our results give support to the role of the expression of angiogenic proteins, such as PECAM, as a plausible mechanism by which Cd^{2+} enhance tissue resistance to ischemia.

Our systematic observations of smaller tissue damage in the Cd^{2+}-treated group after ligature of the left coronary artery, compared with control, suggest that the hearts of exposed animals developed some degree of resistance to ischemia-induced injury, as confirmed by our echocardiographic studies (Fig. 2). The echocardiography performed to control animals after MI indicated a left ventricular

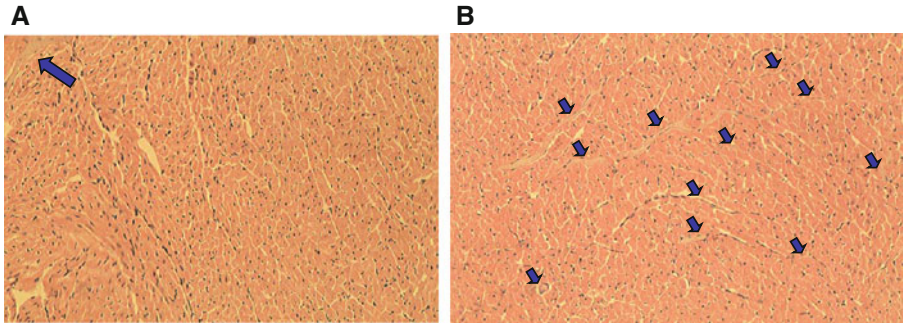

Fig. 5 Histological images of four-micron (4-μm) sections of hearts of control (**a**) and Cd^{2+}-treated adult offspring (**b**) stained with haematoxylin-eosin for morphological analyses. *Arrows* indicate vascularity

hypertrophy and a decreased fractional shortening percentage as result of a decreased contractile capacity (Table 1; Fig. 2). These cardiac alterations were not observed in adult offspring which were exposed to Cd^{2+} during development. In addition, increased levels of plasma cytosolic enzymes like lactate dehydrogenase and glutamic oxalacetic transaminase (Table 2) were found in control animals after MI. The level of these plasma enzymes serve as diagnostic markers of myocardial tissue damage because they reflect alterations in plasma membrane integrity and/or permeability; leak out from the damaged tissues to the blood stream when the cell membrane becomes permeable or rupture [19].

Higher resistance against MI may be due to increased myocardial microvasculature, which translates into a greater number of collaterals [12]. Then, we evaluated whether prenatal Cd^{2+} exposure could have induced the expression of angiogenic factors in the developing fetal heart with long-term consequences. Under similar experimental conditions, we have recently reported that exposed adult female offspring show a reduced endothelial vascular function [8]. In this study, we have performed histological analysis and determined the expression of PECAM and VEGF in adult offspring of both groups before the MI induction because this angiogenic factors are the main mediators of coronary vessel formation in the heart [13, 20, 21]. The histological analysis showed an increased vascularization in hearts of exposed offspring compared with control (Fig. 5). Although we recognize that more specific methods are necessary to confirm a higher vascularization, this preliminary histological study was consistent with an increased expression of the PECAM protein (Fig. 3). The PECAM-1/CD31 is a cell adhesion and signaling receptor molecule essential for angiogenesis that is expressed on haematopoietic and endothelial cells [21]. PECAM-1 is vital to the regulation of inflammatory responses, as it has been shown to serve a variety of pro-inflammatory and anti-inflammatory functions. The potential higher vascularization in the hearts of the Cd^{2+}-treated group induced by PECAM-1 found in our study may be difficult to explain if it had been induced by a direct action of Cd^{2+} on the developing fetus. Most studies with cultured cells directly exposed to Cd^{2+} demonstrated a negative regulation of the production of pro-angiogenic factors or direct anti-angiogenic actions [22, 23]. Conversely, there are few studies revealing paradoxical action of Cd^{2+}, in which PECAM-1 expression was markedly decreased at 48 h but significantly increased at 7 days after Cd^{2+} administration, indicating biphasic changes in PECAM-1 [24].

In our study, we found decreased VEGF protein levels in the cardiac tissue of Cd^{2+}-treated animals. Primary cultured human endometrial cells incubated directly with Cd^{2+} showed inhibition of angiogenic processes through a decrease in the mRNA of VEGF and placental growth factor (PLGF), two important angiogenic molecules [25]. The relationship between VEGF expression, microvasculature, and cardiac function was demonstrated in experiments using transgenic mice lacking the VEGF isoforms VEGF164 and VEGF188. This animal model exhibited impaired myocardial angiogenesis and subsequently developed severe left ventricular (LV) dysfunction [20]. Also, in humans, dilated cardiomyopathy was related to a decreased expression (mRNA and protein) of VEGF and VEHF receptor (VEGFR) that is consistent with the role of VEGF in the myocardial microvasculature [26]. VEGF increases endothelial release of the vasodilator nitric oxide (NO) by the activation of NO synthase. Elevated VEGF concentrations are present in CVD [27], with increased expression in atherosclerotic vessels [28], and are generally associated with improved endothelial function and collateral formation in disease states [29]. In addition, VEGF may be related to the pathogenesis of endothelial dysfunction in healthy smoking individuals. Patients with normal ventricles or history of infarction in the past had no detectable levels of VEGF mRNA [11]. VEGF expression is also substantially increased in chronically ischemic myocardium, as well as immediately after myocardial infarction in the rat heart [30]. In adult tissues and myocardial cell cultures, expression of VEGF is significantly increased by hypoxia [31]. Local expression of VEGF may have an important role in the vascular expansion necessary to form these vascular structures [12], suggesting that in

Table 2 Biochemical measurements

	Control post-infarction	Cd^{2+}-treated post-infarction
Glucose (mg/dl)	179 ± 72	232 ± 41
Ureic Nitrogen (mg/dl)	17 ± 3	13 ± 2
Uric Acid (mg/dl)	2 ± 0.7	0.7 ± 0.1
Calcium (mg/dl)	10 ± 0.5	10.2 ± 0.2
Phosphorous (mg/dl)	8.4 ± 1	7.4 ± 0.3
Total Proteins (g/dl)	5.6 ± 0.2	5.1 ± 0.3
Albumin (g/dl)	2.7 ± 0.2	2.4 ± 0.1
Total Bilirrubin (mg/dl)	0.28 ± 0.1	0.2 ± 0
Glutamic oxalacetic transaminase (OT) (U/l)	147.8 ± 43	73.7 ± 13*
Total alkaline phosphatases (U/l)	300 ± 30	357 ± 69
Total lactic dehydrogenase (LDH)(U/l)	8,121 ± 780	1,178 ± 124*
Electrolytes (mEq/l)		
Sodium	140 ± 5	138.7 ± 0.6
Potassium	7.2 ± 3	4.2 ± 0.3
Chloride	100 ± 1	103.7 ± 0.6
Lipid profile (mg/dl)		
Total cholesterol	71.8 ± 6	69.7 ± 13
HDL cholesterol	39.3 ± 3	37.3 ± 8
LDL cholesterol	26.3 ± 5	25.9 ± 4
Triglycerides	31.3 ± 18	32.3 ± 10
Risk factor	1.8 ± 0.2	1.9 ± 0.2
Creatinine (mg/dl)	0.6 ± 0.1	0.5 ± 0.1
Glomerular filtration Vel (ml/min)	175 ± 28	199 ± 23
Troponin (ng/ml)	0.01 ± 0	0.01 ± 0
Protein C reactive (ultrasensitive) (mg/l)	0.1 ± 0	0.1 ± 0

Values are means ± SEM of 6 determinations; * $P < 0.05$ compared with respective control of $n = 6$ samples, Mann–Whitney U test

our study this pathway has not been involved in the changes induced by the Cd^{2+} treatment during fetal cardiac development.

The dose of prenatal Cd^{2+} exposure used in this study was not high enough to be transferred to the fetal tissues as we recently reported [8], and, in consequence, probably Cd^{2+} is not directly acting on the developmental organs, but rather it accumulates in the placenta to induce adaptive mechanisms in the developing fetus. There are no data on the literature reporting effects on cardiac function in adult offspring which were exposed to Cd^{2+} during gestation; most studies reporting effects of Cd^{2+} on cardiac function have been performed by a direct exposure of animals to Cd^{2+}, and from this point of view, both types of studies may be not comparable.

We have recently reported that prenatal Cd^{2+} treatment induced an overexpression of HO-1 mRNA expression in aorta of adult offspring [8]. It has been demonstrated that HO-1 is an inducer of VEGF expression in heart tissue, and so we expected to measure a higher expression of VEGF [32]. Although Cd^{2+} exposure dose were similar in both studies (30 ppm of Cd^{2+}), we can argue that both responses could be related with the different tissues in which VEGF and HO-1 were determined (heart or aorta).

In humans, recent studies in offspring of mothers who smoked during pregnancy reported that maternal smoking is associated with increased rise in total cholesterol levels and a tendency toward an adverse lipoprotein profile in the young offspring [33]. Also, in the 1958 British birth cohort, offspring of smoking mothers registered higher markers of cardiovascular risk, such as increased body mass index (BMI) and higher blood pressure and triglycerides [34]. Although several chemicals contained in tobacco smoke could mediate those effects, in humans, Cd^{2+} trace levels have been detected in umbilical cords indicating that although the placenta is an effective barrier against Cd^{2+} transport to the fetus, it can cross and reach the fetus inducing direct deleterious effect [35, 36]. In epidemiological studies, high Cd^{2+} levels in blood and urine have been directly associated with high intima media thickness [37], increased prevalence of peripheral arterial disease (PAD) [38] and higher blood pressure [39]. Also, tobacco smoking is one of the most important causes of MI globally, especially in men [40]. Therefore, all epidemiological data collected until now in humans show that Cd^{2+} levels in body burden constitute a risk of CVD. Thus, the potential protective effects of Cd^{2+} exposure during development at long time found in our study may be rather indirect than a

direct effect of Cd^{2+} on the developing fetus. This hypothesis is supported by our previous findings in which maternal exposure to 30 ppm of Cd^{2+} does not cross the placental barrier in rats; we only detected Cd^{2+} in placental tissues but not in any of the analyzed fetal tissues [8]. A different possibility is that protective effect may be specific for rodents and may be not be applicable to humans.

In summary, this study demonstrates effectively a reduced infarct size and an improvement in cardiac function after experimental myocardial infarction performed in offspring adult rats that were exposed to Cd^{2+} (30 ppm) during development. The protection of prenatal exposure to Cd^{2+} against MI at adult age may be associated with increased cardiac angiogenesis that occurred during prenatal development, which may have induced an increase in the capillary density and PECAM protein level, thus providing protection against ischemia. In addition, our previous results showing increased expression of HO-1 in aortas of adult female which were exposed to Cd^{2+} during pregnancy may have contributed to alleviating the effects of ischemia in the cardiac tissue after MI. This fundamental observation may serve as a basis in locally targeting angiogenic activators in high-risk MI individuals. Finally, this study reveals once again the innumerable paradoxical effects mediating the biological actions of Cd^{2+}.

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