Combination Therapy for the Cardiovascular Effects of Perinatal Lead Exposure in Young and Adult Rats

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Abstract

Background: Combination therapy can play a significant role in the amelioration of several toxic effects of lead (Pb) and recovery from associated cardiovascular changes.

Objective: To investigate the effects of combination therapy on the cardiovascular effects of perinatal lead exposure in young and adult rats

Methods: Female Wistar rats received drinking water with or without 500 ppm of Pb during pregnancy and lactation. Twenty-two- and 70-day-old rat offspring who were or were not exposed to Pb in the perinatal period received meso-dimercaptosuccinic acid (DMSA), L-arginine, or enalapril and a combination of these compounds for 30 additional days. Noradrenaline response curves were plotted for intact and denuded aortas from 23-, 52-, 70-, and 100-day-old rats stratified by perinatal Pb exposure (exposed/unexposed) and treatment received (treated/untreated).

Results: Systolic blood pressure was evaluated and shown to be higher in the 23-, 52-, 70-, and 100-day age groups with Pb exposure than in the corresponding control age groups: 117.8 ± 3.9*, 135.2 ± 1.3*, 139.6 ± 1.6*, and 131.7 ± 2.8*, respectively and 107.1 ± 1.8, 118.8 ± 2.1, 126.1 ± 1.1, and 120.5 ± 2.2, respectively ($p < 0.05$). Increased reactivity to noradrenaline was observed in intact, but not denuded, aortas from 52-, 70-, and 100-day-old exposed rats, and the maximum responses (g of tension) in the respective Pb-exposed and control age groups were as follows: 3.43 ± 0.16*, 4.32 ± 0.18*, and 4.21 ± 0.23*, respectively and 2.38 ± 0.33, 3.37 ± 0.13, and 3.22 ± 0.21, respectively ($p < 0.05$).

Conclusions: All treatments reversed the changes in vascular reactivity to noradrenaline in rats perinatally exposed to Pb. The combination therapy resulted in an earlier restoration of blood pressure in Pb-exposed rats compared with the monotherapies, except for enalapril therapy in young rats. These findings represent a new approach to the development of therapeutic protocols for the treatment of Pb-induced hypertension. (Arq Bras Cardiol. 2014; 103(3):219-230)

Keywords: Perinatal Exposure; Cardiovascular Effects; Lead / toxicity; Rats.

Introduction

Epidemiological data show that lead (Pb) plays a role in the development of arterial hypertension in individuals occupationally exposed to Pb and in the general population[1].

Arterial hypertension induced by Pb exposure during postnatal life is characterized by an increase in vascular reactivity to catecholamines[2], a decrease in beta-adrenergic receptors[3], and a decrease in vasodilatory responses to acetylcholine and sodium nitroprusside[4]. In contrast, Purdy et al[5] reported no changes in aortic reactivity to either vasoconstrictors (noradrenaline and phenylephrine) or vasodilators (acetylcholine and sodium nitroprusside) associated with for Pb-induced hypertension.

Although great progress has been made with regard to neurochemical and behavioral alterations induced by perinatal Pb exposure[6,7], the cardiovascular effects of Pb exposure remain unclear.

Studies have focused on the concept that Pb is associated with regulatory processes involving Ca^{+2}, cyclic GMP, and protein kinase C[1,8,9]; renin–angiotensin–aldosterone, kallikrein–kinin, and other autacoidal (e.g., endothelin) and transductional systems [e.g., nitric oxide (NO)][1,8,10]; endothelium and smooth muscle proliferation[11,12]; and oxidative stress[13].

Despite many years of research, the optimal treatment for toxicity caused by heavy metal exposure remains to be determined. Chelation therapy is the preferred treatment for decreasing the toxic effects of metals, although chelators have largely been restricted to overt, acute poisoning[14,15]. Several supplements such as vitamins, sulfur-containing amino acids,

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antioxidants, and essential minerals are also used to address metal toxicity[15]. Combination therapy is a novel and better approach to the treatment of metal poisoning[14,15]. Because little experimental evidence is available, there is a requirement for in-depth investigations in this field.

On the basis of the abovementioned reports, we investigated combination therapies for the treatment of Pb-induced cardiovascular toxicity. The effects of maternal Pb exposure during pregnancy and lactation on blood pressure and aortic reactivity were investigated in young and adult rat offspring, with focus on endothelial and smooth muscle cells. We then evaluated the therapeutic effects of the following drugs administered either alone or in combination on the adverse cardiovascular effects of perinatal Pb exposure in young and adult rats: meso-dimercaptosuccinic acid (DMSA), a chelating agent and scavenger of reactive oxygen species (ROS)[16,17]; L-arginine, a precursor of NO[18]; and enalapril, an angiotensin-converting enzyme inhibitor.

Methods

Animals and Pb exposure

Adult Wistar rats were obtained from University of São Paulo facilities and used as the parent generation. The animals were mated at the age of 90 days (two females and one male per cage). On pregnancy day 0 (determined by the presence of sperm in vaginal smears), the females were divided into nonexposed and Pb-exposed groups and were housed alone. The drinking water in the cages housing the females was altered with 500 ppm Pb (as Pb acetate) or tap water. A group of animals received sodium acetate to equalize acetate exposure between groups. The Pb exposure regimen was chosen on the basis of previous studies[6,7,19]. To prevent the formation of a Pb precipitate, 0.5 mL of glacial acetic acid was added while stirring to 1000 mL of each solution (sodium acetate and Pb). Pb exposure lasted throughout pregnancy and lactation (Figure 1). At birth, the number of pups per litter was recorded, following which all litters were culled to eight pups. Whenever possible, only male rats were kept within the litter, and females were kept just to maintain equal litter sizes. Pups were weaned at 22 days of age on tap water and evaluated at 23 and 70 days of age (Figure 1). Age-matched controls received sodium acetate during the same periods as that of Pb exposure. Maternal body weights were measured on pregnancy day 0, the day before delivery, after delivery, and at weaning. Pup weights were recorded at birth and weekly until 100 days of age.

Lights in the animal room were set on a 12:12-h light-dark cycle, with the temperature maintained at 22 ± 1°C. The animals were fed with regular laboratory chow. Animal procedures were performed in accordance with the principles and guidelines of the National Council for Control of Animal Experimentation (protocol no. 25/05-CEEA).

Measurement of blood pressure

Beginning at 22 days of life, systolic blood pressure was determined weekly in conscious rats using the tail-cuff plethysmographic method (Narco Bio-Systems, Inc., Houston, TX). The rats were prewarmed for approximately 10 min and placed into a restrainer for blood pressure measurement. Three consecutive recordings (approximately 1 min apart) were obtained, and the mean of these three measurements was recorded.

Determination of Pb levels in blood

Whole blood was collected from the hearts of anesthetized (urethane, 1.25 g/kg) female rats at weaning and pups at 23, 52, 70, and 100 days of age.

Blood was prepared for Pb analysis, which was conducted using a microwave dissolution procedure utilizing a DGT-100 plus microwave digestion apparatus (Provecto, Brazil). Nitric acid was added to the digestion vessels containing 1 mL of blood.

Pb levels in whole blood were determined using an atomic absorption spectrophotometer GBC AA 932 (EEA-flame)[20]. The recovery of Pb that was externally added to control samples was found to be consistently greater than 96%. A standard addition method was applied during determination to eliminate possible matrix interference. Whole-blood Pb levels were expressed in micrograms per deciliter. The detection limit was 5 μg/dL.

Therapy

A group of 22- and 70-day-old rats who were or were not exposed to Pb in the perinatal period received DMSA, L-arginine, enalapril, or the combination of these compounds for 30 additional days (Figure 1). DMSA was orally administered at 60 mg/kg by gastric gavage two times a day (30 mg/kg per dose) for 5 days a week. L-arginine (1.0%) was administered through drinking water consumed *ad libitum*. Enalapril was also administered through drinking water (approximately 5 mg/day/rat). Age-matched controls received tap water.

Experimental protocols

Immediately after the blood samples were collected, the thoracic aorta was excised and trimmed free of adhering fat and connective tissue. Two transverse rings measuring approximately 4 mm in length were cut and mounted at the optimal length for recording isometric tension in organ chambers. One ring served as a control, while the endothelium was mechanically removed from the other by gently rubbing the luminal surface. These samples constituted the intact and denuded aortas, respectively. The organ baths contained Krebs–Henseleit solution (7 mL) comprising (in mM) NaCl (113.0), KCl (4.7), $CaCl_2$ (2.5), $NaHCO_3$ (25.0), $MgSO_4$ (1.1), KH_2PO_4 (1.2), ascorbic acid (0.11), and glucose (11.1). The bathing fluid was maintained at 37°C and was saturated with a gas mixture of 95% O_2 and 5% CO_2. The preparations were allowed to equilibrate for at least an hour under a resting tension of 1.5 g, which is optimal for inducing maximum contraction. Tension was recorded using an F-60 microdisplacement myograph (Narco Bio-Systems Inc., Houston, Texas, USA) and displayed on a physiograph. Intact and denuded aortas from rats who were or were not exposed to Pb were studied in parallel.

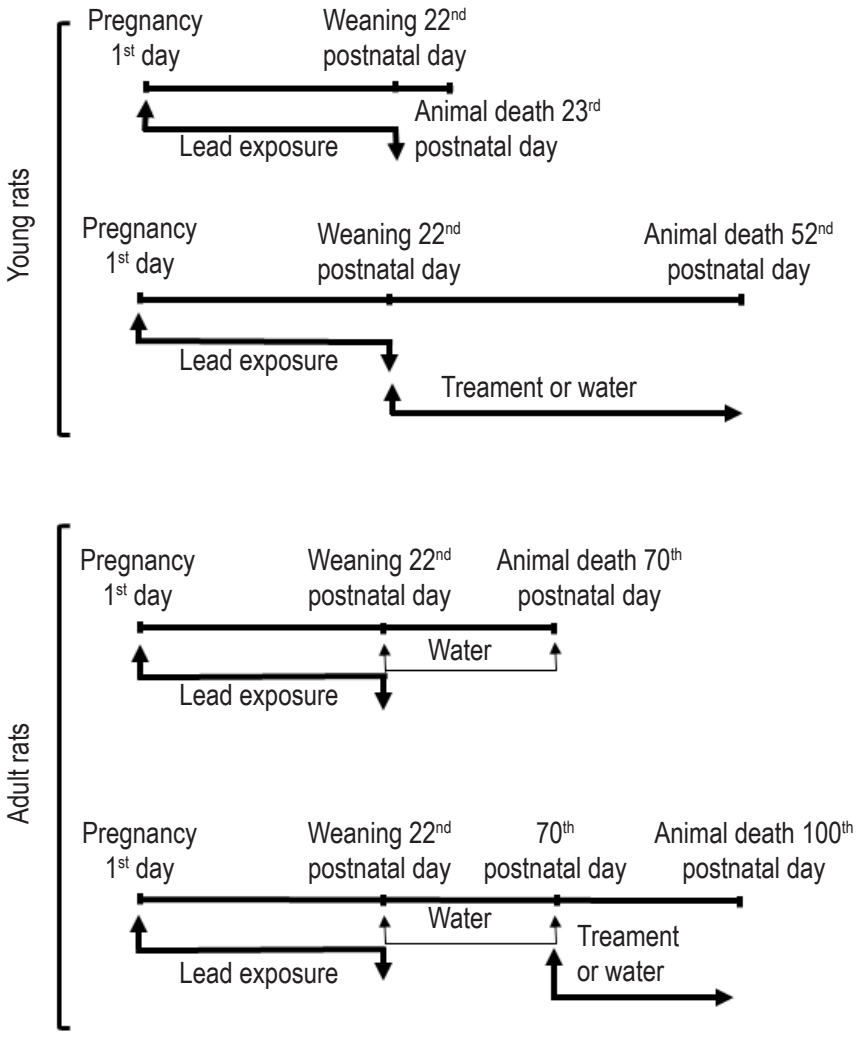

Figure 1 – *Experimental design.*

Cumulative concentration-effect curves were constructed from the aortic response to noradrenaline. At the ends of the curves, acetylcholine (10^{-6} M) and sodium nitroprusside (10^{-4} M) were used to test the integrity of the endothelial and smooth muscle layers, respectively.

Drugs and solutions

The following drugs were used: acetylcholine bromide, DMSA, enalapril maleate, L-arginine, lead acetate, noradrenaline bitartrate, sodium nitroprusside, and urethane (all obtained from Sigma Chemical Co., St Louis, Missouri, USA). All drugs were dissolved in Krebs–Henseleit solution, and the concentrations were expressed in molarity. Lead and sodium acetate were dissolved in acidified tap water as described above. L-arginine and enalapril were dissolved in tap water. DMSA solutions were freshly prepared by dissolving the compound in 5% $NaHCO_3$.

Data analysis and statistics

The litter was considered to be the experimental unit in all performed analyses. The concentration of vasoactive agents producing a response that was 50% of the maximum (EC_{50}) was calculated in each experiment. The EC_{50}s to noradrenaline are presented as means with 95% confidence intervals. The maximal response to noradrenaline is presented as means ± standard errors (SEs). Blood pressure, EC_{50} values, and maximal responses were compared by two-way analysis of variance using SigmaStat

3.2 software. Pb and treatment were the factors included in the analysis. A P-value of <0.05 was considered statistically significant. Tukey's multiple comparisons test was used to test differences among means. Other parameters (body weight, number of pups, and Pb determination) are also presented as means ± standard errors and were compared using the Mann–Whitney test, which was conducted using InStat 4.0 software.

Results

Body weight, number of pups, blood Pb levels, and blood pressure

Pb exposure did not affect the body weight of the mothers and pups (Table 1) as well as the number of pups per litter (control: 11.3 ± 0.4, Pb: 10.5 ± 0.5, $p > 0.05$; number of litters per group, 10–20).

Pb levels in blood samples from the mothers and the 23-, 52-, 70- and 100-day-old rats were significantly higher than those in the respective controls (Table 2). After Pb discontinuation, the blood Pb levels in exposed rats decreased but remained higher than those in control rats (Table 2). The different treatment protocols resulted in a significant decrease in blood Pb levels in the 52- and 100-day-old rats (Table 2). These decreases reached values similar to those observed in control rats of the same age, except in rats treated with L-arginine, in which blood Pb levels remained high compared with those in controls (Table 2).

Systolic blood pressure was not altered by sodium acetate exposure compared with those after tap water exposure (data not shown). Systolic blood pressure showed a gradual increase during postnatal development (Figures 2 and 3). Moreover, the values observed in Pb-exposed rats were significantly higher than those in controls (Figures 2 and 3).

All treatments decreased the blood pressure of Pb-exposed young rats to levels near those in unexposed animals. This reversal in blood pressure occurred at the age of 49, 35, 28, and 28 days, respectively, with L-arginine, DMSA, enalapril, and combination therapy (Figure 2). Similar reversals in blood pressure were observed in Pb-exposed adult rats treated with L-arginine, enalapril, and combination therapy at the age of 84, 84, and 77 days, respectively (Figure 3). Although DMSA treatment decreased blood pressure in Pb-exposed adult rats, this value was similar to that observed in both unexposed and Pb-exposed untreated rats (Figure 3).

Vascular reactivity

The reactivity of intact and denuded aortas in Pb-exposed rats was not altered by sodium acetate exposure compared with that in aortas from rats that received tap water (data not shown). Gavage did not cause any change in aortic reactivity (data not shown). Neither Pb exposure nor the treatments altered the reactivity of denuded aortas to noradrenaline (Tables 3 and 4).

Removal of the endothelium caused a leftward shift of the noradrenaline response curve, which was similar in aortas from both controls and Pb-exposed rats, irrespective of treatment received or not (Tables 3 and 4). This procedure also resulted in an increase in the maximum response to noradrenaline in aortas from different experimental groups (Tables 3 and 4). After removal of the endothelium, the maximum response to noradrenaline was similar in the aortas from all experimental groups (Tables 3 and 4).

With regard to intact aortas, no change was observed in the maximum response to noradrenaline in 23-day-old pups exposed to Pb in the perinatal period (Table 3). In contrast, an increase in the reactivity of intact aortas was observed in 52-, 70-, and 100-day-old rats exposed to Pb in the perinatal period (Table 3). Independent of the protocol, the noradrenaline sensitivity of aortas with intact endothelium did not differ (Tables 3 and 4).

The different treatments did not alter the reactivity of intact aortas from nonintoxicated rats to noradrenaline (Tables 3 and 4). However, L-arginine, DMSA, enalapril, and combination therapy were effective in decreasing the Pb-induced increase in maximum aortic response to levels similar to those in the respective controls (Tables 3 and 4).

Discussion

Public health authorities use high levels to define blood Pb levels of concern in nonpregnant women, with 40 μg/dL being the adult reference value in many countries.

We must recognize that a significant proportion of nonpregnant women with blood Pb levels ≥ 40 μg/dL can become pregnant and potentially expose their infants to the risk of adverse health effects caused by Pb[21]. Maternal and fetal blood Pb levels are nearly identical because Pb crosses the placenta unencumbered. This has provoked a concern about increased blood Pb levels among all females of childbearing age because a great proportion of pregnancies are unplanned. Finally, pregnant women without symptoms frequently remain in contact with the source of exposure during pregnancy and lactation, particularly in developing countries.

The severity of effects and the extent to which the cardiovascular system is affected by Pb appear to be influenced most directly by Pb levels, duration of Pb exposure, and other factors such as the route of exposure and individual life phase[22,23]. In the present work, we investigated cardiovascular alterations in rats exposed to Pb *in utero* and during lactation that were born to females with blood Pb levels of approximately 50 μg/dL. The Pb exposure regimen had no effect on the weight of the females and the pups at birth, at weaning, and during postnatal life or on the number of pups per litter. All animals appeared healthy, and none showed signs of toxicity.

However, the Pb exposure protocol resulted in an increase in systolic blood pressure in association with changes in vascular reactivity. The sustained increase in blood pressure observed in weaned, young and adult rats perinatally exposed to Pb confirms the results of previous reports that showed a positive association between blood Pb levels and arterial hypertension[23,24].

Investigation of the manner in which Pb affects vascular endothelial and smooth muscle cells was the goal of the present study. Changes in the balance of endothelial contracting and

Table 1 – Body weights of females exposed or not exposed to Pb during pregnancy and lactation and their male offspring who were treated or not treated with DMSA, L-arginine, enalapril, or combination therapy

		Body weight (g)						
Rats	Groups	Pregnancy		Lactation		Post-weaning age		
		0 day	21 days	1 day	21 days	52 days	70 days	100 days
Dams	Control (n = 20)	272.2 ± 6.2	378.8 ± 9.0	304.6 ± 7.0	276.9 ± 5.6	-	-	-
	Pb (n = 20)	269.0 ± 4.9	360.3 ± 8.1	293.1 ± 5.0	275.44 ± 5.3	-	-	-
Male offspring	Control (n = 20)	-	-	6.2 ± 0.2	39.1 ± 0.5	208.3 ± 0.2	323.1 ± 5.3	357.0 ± 2.7
	Pb (n = 20)	-	-	5.8 ± 1.7	40.0 ± 1.2	198.6 ± 4.1	327.9 ± 8.6	357.1 ± 3.2
	Pb/DMSA (n = 20)	-	-	-	41.2 ± 2.2	195.0 ± 2.2	326.8 ± 6.1	365.7 ± 1.7
	Pb/L-arginine (n = 15)	-	-	-	43.0 ± 2.3	205.5 ± 2.3	325.4 ± 2.5	369.9 ± 2.2
	Pb/enalapril (n = 15)	-	-	-	42.4 ± 1.8	194.8 ± 4.2	301.7 ± 2.4	351.1 ± 1.8
	Combination therapy (n = 20)	-	-	-	43.0 ± 5.5	206.0 ± 8.5	314.7 ± 2.6	360.6 ± 1.2

Values represent means ± standard errors. Pb: 500 ppm lead acetate during pregnancy and lactation. Treatment: a group of 22- and 70-day-old offspring who were or were not exposed to Pb during the perinatal period received DMSA, L-arginine, enalapril, or a combination of these compounds for 30 additional days. DMSA was orally administered at 60 mg/kg by gastric gavage two times a day (30 mg/kg per dose) for 5 days a week. L-arginine (1.0%) was administered through drinking water ad libitum. Enalapril was administered through drinking water at a dosage of 5 mg/rat/day. Age-matched controls received tap water. (n) = number of animals per group.

Table 2 – Blood Pb levels in females who were or were not exposed to Pb during pregnancy and lactation and their male offspring who were or were not treated with DMSA, L-arginine, enalapril, or combination therapy

	Blood Pb levels (µg/dL)				
Groups	Females	Male offspring			
		23 days	52 days	70 days	100 days
Control (n = 5)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Pb (n = 6)	53.39 ± 5.88*	35.61 ± 7.43*	-	-	-
Pb/water (n = 6)	-	-	19.98 ± 6.31*	13.15 ± 0.97*+	11.17 ± 2.11*+
Pb/DMSA (n = 5)	-	-	< 5.0$	-	< 5.0$
Pb/L-arginine (n = 6)	-	-	10.65 ± 2.12*	-	6.55 ± 3.29*
Pb/enalapril (n = 5)	-	-	< 5.0$	-	< 5.0$
Combination therapy (n = 5)	-	-	< 5.0$	-	< 5.0$

*Values represent means ± standard errors. Pb: 500 ppm lead acetate during pregnancy and lactation. Treatment: a group of 22- and 70-day-old offspring who were or were not exposed to Pb during the perinatal period received DMSA, L-arginine, enalapril, or a combination of these compounds for 30 additional days. DMSA was orally administered at 60 mg/kg by gastric gavage two times a day (30 mg/kg per dose) for 5 days a week. L-arginine (1.0%) was administered through drinking water ad libitum. Enalapril was administered through drinking water at a dosage of 5 mg/rat/day. Age-matched controls received tap water. *p < 0.05 compared with the respective control group; +p < 0.05 compared with Pb-exposed, 23-day-old rats; $p < 0.05 compared with the respective Pb/water group; (n) = number of animals in each age group.*

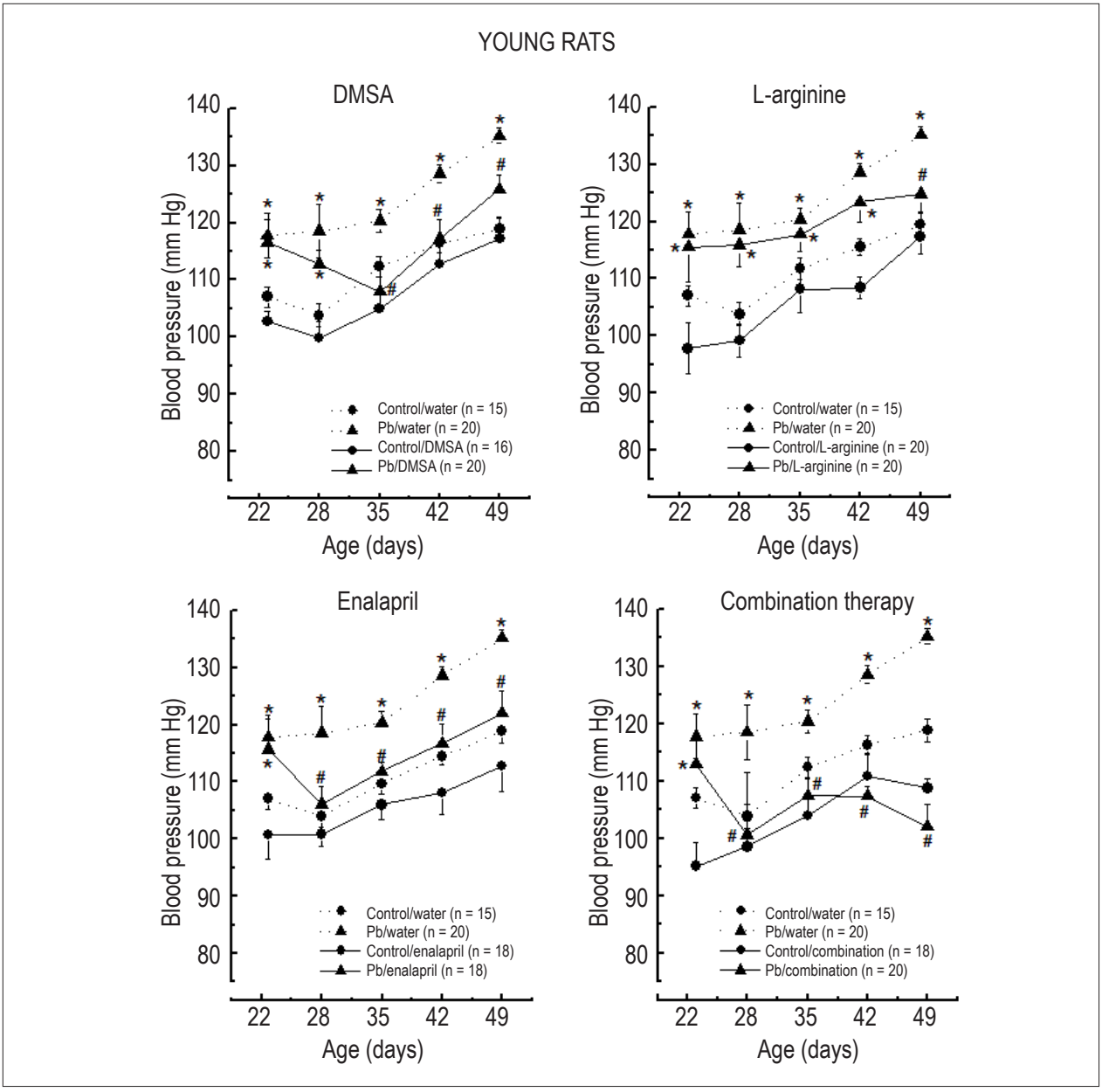

Figure 2 – *Arterial blood pressure in 52-day-old rats who were or were not exposed to Pb in the perinatal period (during pregnancy and lactation) and were treated or not treated with DMSA, L-arginine, enalapril, or combination therapy. Treatment: a group of 22-day-old offspring received DMSA, L-arginine, enalapril, or a combination of these compounds for 30 additional days. DMSA was orally administered at 60 mg/kg by gastric gavage two times a day (30 mg/kg per dose) for 5 days a week. L-arginine (1.0%) was administered through drinking water ad libitum. Enalapril was administered through drinking water at a dosage of 5 mg/rat/day. Age-matched controls received tap water. Values are expressed as means ± standard errors. *p < 0.05 compared with controls; #p < 0.05 compared with Pb/water. (n) = animal number per group.*

relaxing factors contribute to arterial hypertension associated with Pb poisoning. A decrease in NO, which is a vasodilator component, and an increase in endothelin-3 and natriuretic hormone, which are vasoconstrictor components, have been reported to be responsible for arterial hypertension in rats exposed to low levels of Pb[8,25-27]. Our findings corroborate these reports because arterial hypertension induced by perinatal Pb exposure was shown to be associated with an increase in aortic reactivity to noradrenaline. Moreover, this hyperreactivity was a consequence of endothelial cell dysfunction because removal of the endothelium abolished this effect.

Another interesting observation was the time-dependent expression of perinatal Pb exposure-induced changes in vascular reactivity in postnatal life. Fifty-two-, 70-, and 100-day-old, but not 23-day-old, rats exhibited these changes. It is likely that, at this age, the hypertensive state induced by perinatal Pb exposure was due to the presence of circulating factor(s) and hemodynamic changes.

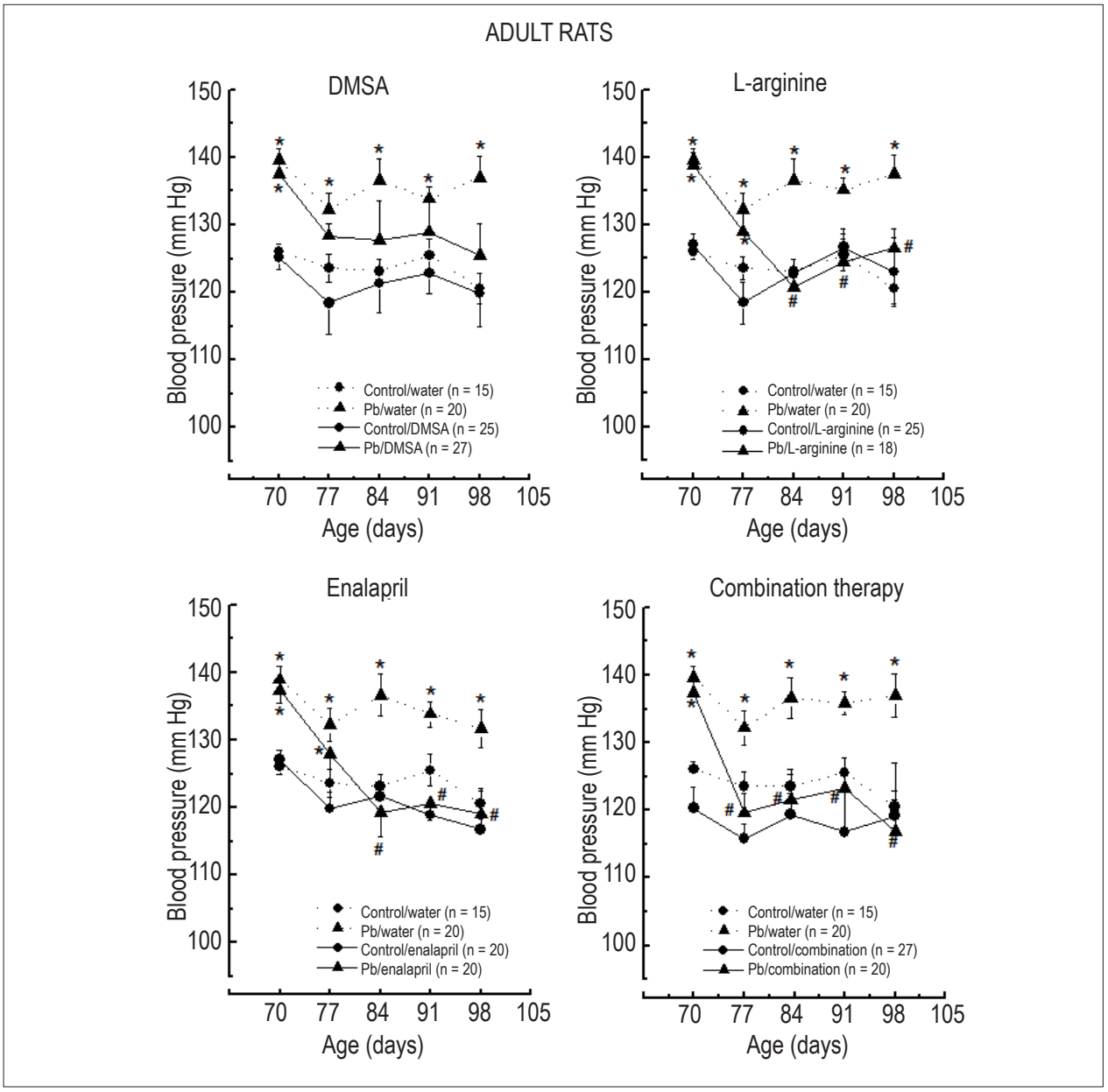

Figure 3 – *Arterial blood pressure in 100-day-old rats who were or were not exposed to Pb in the perinatal period (during pregnancy and lactation) and were treated or not treated with DMSA, L-arginine, enalapril, or combination therapy. Treatment: a group of 100-day-old offspring received DMSA, L-arginine, enalapril, or a combination of these compounds for 30 additional days. DMSA was orally administered at 60 mg/kg by gastric gavage two times a day (30 mg/kg per dose) for 5 days a week. L-arginine (1.0%) was administered through drinking water ad libitum. Enalapril was administered through drinking water at a dosage of 5 mg/rat/day. Age-matched controls received tap water. Values are expressed as means ± standard errors. *p < 0.05 compared with controls; #p < 0.05 compared with Pb/water; (n) = animal number per group.*

Vascular smooth muscle alterations in Pb-induced hypertension have also been reported in the literature and were shown to be associated with an increase in intracellular calcium[9,22], an interaction with protein Kinase C[9], the inhibition of Na^+/K^+-ATPase)[28,29], and an inhibitory or stimulatory effect on various blood pressure-related humoral factors[8]. However, no smooth muscle changes were observed in aortas from young or adult rats perinatally exposed to Pb. This controversy could be due to differences in the Pb exposure protocols related to doses and/or duration of exposure.

Finally, other reported mechanisms by which Pb could contribute to the development of arterial hypertension include an increase in the activity of angiotensin-converting enzyme and an increase in the levels of plasma renin, angiotensin II, aldosterone, and kininases[1].

The ongoing and widespread problem of Pb intoxication is treated by first isolating the individual from the Pb-contaminated environment, followed by administering a Pb-chelating agent. However, a new trend in toxic metal therapy has recently

Table 3 – Maximal responses and EC50 values for the response to noradrenaline obtained in aortas with and without endothelium that were isolated from rats who were or were not exposed to Pb in the perinatal period (during pregnancy and lactation) and were or were not treated with L-arginine or enalapril

Male offspring		Aorta With Endothelium			Aorta Without Endothelium		
		[1]Maximum response (g of tension)	[2]EC_{50} ($\times 10^{-8}$ M)	[3]N	[1]Maximum response (g of tension)	[2]EC_{50} ($\times 10^{-9}$ M)	[3]N
Not treated							
23-day-old	Control	1.8 ± 0.1	1.3 (0.5 – 3.4)	6	2.6 ± 0.2^{+}	1.4^{+} (0.3 – 6.4)	8
	Pb	2.1 ± 0.2	1.5 (0.4 – 4.9)	6	3.0 ± 0.1^{+}	1.1^{+} (0.1 – 7.5)	8
52-day-old	Control	2.4 ± 0.3	2.0 (0.4 – 10.8)	7	4.1 ± 0.4^{+}	1.0^{+} (2.8 – 6.4)	8
	Pb	3.4 ± 0.2*	11.0 (6.0 – 20.3)	6	4.5 ± 0.2^{+}	1.0^{+} (0.3 – 4.2)	8
70-day-old	Control	3.4 ± 0.1	16.0 (7.1 – 35.4)	5	5.2 ± 0.4^{+}	5.0^{+} (0.7 – 36.0)	7
	Pb	4.3 ± 0.2*	4.8 (1.7 – 13.3)	6	5.0 ± 0.3^{+}	1.3^{+} (0.4 – 4.2)	8
100-day-old	Control	3.2 ± 0.2	8.5 (4.6 – 15.6)	6	5.3 ± 0.3^{+}	5.6^{+} (1.4 – 21.9)	8
	Pb	4.2 ± 0.2*	4.3 (0.9 – 20.7)	6	5.2 ± 0.4^{+}	3.8^{+} (0.7 – 19.7)	8
L-arginine treatment							
52-day-old	Control	2.8 ± 0.1	3.3 (1.5 – 7.3)	5	4.5 ± 0.3^{+}	1.6^{+} (0.1 – 29.0)	8
	Pb	2.7 ± 0.1	10.0 (4.1 – 37.3)	6	4.3 ± 0.3^{+}	8.5^{+} (1.0 – 27.5)	8
100-day-old	Control	3.3 ± 0.2	8.9 (5.5 -14.5)	8	4.8 ± 0.2^{+}	9.2^{+} (3.2 – 26.3)	8
	Pb	3.2 ± 0.4	8.6 (2.3 – 31.4)	12	4.9 ± 0.5^{+}	0.9^{+} (0.2 – 7.3)	14
Enalapril treatment							
52-day-old	Control	2.7 ± 0.4	3.0 (0.4 – 23.4)	12	4.2 ± 0.3^{+}	0.4^{+} (0.1 – 8.7)	12
	Pb	2.7 ± 0.2	6.15 (1.31 – 28.91)	7	4.2 ± 0.2^{+}	6.2^{+} (1.0 – 38.0)	8
100-day-old	Control	3.1 ± 0.2	5.4 (1.0 -27.5)	8	5.0 ± 0.4^{+}	4.1^{+} (0.6 – 25.9)	11
	Pb	3.2 ± 0.1	9.4 (3.0 – 30.1)	5	5.6 ± 0.6^{+}	1.2^{+} (0.2 – 19.3)	14

[1]Values represent means ± standard errors. [2]EC50: levels producing half-maximum responses; values represent means with 95% confidence intervals. [3]N = number of animals. Pb: 500 ppm lead acetate during pregnancy and lactation. Treatment: a group of 22- and 70-day-old offspring who were or were not exposed to Pb during the perinatal period received received L-arginine or enalapril for 30 additional days. L-arginine (1.0%) was administered through drinking water ad libitum. Enalapril was administered through drinking water at a dosage of 5 mg/rat/day. Age-matched controls received tap water.$p < 0.05$ compared with the respective control group; $^{+}p < 0.05$ compared with the respective aorta with endothelium.*

emerged, where combination therapy is used instead of monotherapy with chelating agents[13,14,26,30]. Therefore, amino acid supplementation during chelation therapy has been found to be beneficial in increasing metal mobilization and facilitating recovery in the presence of several altered physiological variables[24,30].

DMSA is a water-soluble compound that forms a strong complex with Pb^{2+} in the blood, which is subsequently secreted via the kidney[31]. Sulfur-containing amino acids such as methionine and cysteine and metabolically related amino acids increase the bioavailability of glutathione, which is useful in chelating Pb. This counteracts the toxic effects of the metal, potentially making these amino acids useful for supportive therapy[32,33]. Moreover, in metalloregulatory proteins, metals are often conveniently located at binding sites and are bound to cysteine residues. Several lines of evidence indicate that

Table 4 – Maximal responses and EC_{50} values for the response to noradrenaline in aortas with and without endothelium that were isolated from rats who were or were not exposed to Pb during pregnancy and lactation and were or were not treated with DMSA or combination therapy

Male offspring		Aorta With Endothelium			Aorta Without Endothelium		
		[1]Maximum response (g of tension)	[2]EC_{50} (x 10^{-8} M)	[3]N	[1]Maximum response (g of tension)	[2]EC_{50} (x 10^{-9} M)	[3]N
Not treated							
52-day-old	Control	2.8 ± 0.4	9.0 (3.6 – 22.9)	8	4.1 ± 0.4+	12.8+ (1.3 – 93.3)	8
	Pb	4.0 ± 0.4*	11.5 (5.3 – 25.1)	8	4.8 ± 0.2+	2.7+ (0.4 – 20.2)	6
100-day-old	Control	2.7 ± 0.3	7.9 (2.2 – 28.0)	5	5.1 ± 0.4+	10.5+ (0.3 – 49.1)	7
	Pb	4.1 ± 0.2*	15.4 (2.8 – 31.5)	5	5.4 ± 0.4+	15.2+ (0.4 – 69.6)	12
DMSA treatment							
52-day-old	Control	2.8 ± 0.3	13.7 (5.7 – 32.7)	6	4.5 ± 0.3+	13.9+ (2.4 – 81.0)	7
	Pb	2.7 ± 0.3	9.1 (3.8 – 21.8)	6	4.3 ± 0.4+	1.7+ (0.1 – 67.1)	12
100-day-old	Control	2.3 ± 0.2	18.1 (4.4 – 43.8)	6	4.6 ± 0.4+	35.1+ (3.6 – 141.2)	7
	Pb	2.8 ± 0.2	27.6 (11.6 – 55.6)	6	4.3 ± 0.5+	11.8+ (0.1 – 151.7)	14
Combination therapy							
52-day-old	Control	3.1 ± 0.2	7.1 (3.7 – 13.5)	5	4.9 ± 0.3+	0.3+ (0.1 – 17.8)	6
	Pb	2.4 ± 0.3	5.0 (2.8 – 9.1)	6	3.9 ± 0.6+	10.1+ (0.1– 23.3)	14
100-day-old	Control	3.2 ± 0.3	7.0 (2.2 -22.5)	7	4.8 ± 0.3+	4.7+ (1.2 – 18.0)	6
	Pb	2.8 ± 0.2	9.7 (1.6 – 30.3)	6	4.4 ± 0.3+	6.2+ (0.1 – 39.7)	8

*[1]Values represent means ± standard errors. [2]EC50: levels producing half-maximum responses; values represent means with 95% confidence intervals. [3]N = number of animals. Pb: 500 ppm lead acetate during pregnancy and lactation. Treatment: a group of 22- and 70-day-old offspring who were or were not exposed to Pb in the perinatal period received DMSA or combination therapy for 30 additional days. The combination therapy included L-arginine (1.0%, in drinking water) + enalapril (5 mg/day/rat, in drinking water) + DMSA (60 mg/kg/day). DMSA was orally administered at 60 mg/kg by gastric gavage two times a day (30 mg/kg per dose) for 5 days a week. Aged matched-controls received tap water. *$p < 0.05$ compared with the respective control group; +$p < 0.05$ compared with the respective aorta with endothelium.*

cysteine-rich metal-binding proteins as well as redox-sensitive metal clusters of metalloproteins are natural sensors of bioradicals such as NO[34]. In fact, Misra et al[35] showed that NO mediates cadmium release from metallothionein. Therefore, an increase in NO levels by L-arginine treatment may displace Pb from its cellular binding sites, as previously reported by Malvezzi et al[24].

Corroborating previous data from our laboratory[24], the Pb burden in the body was significantly decreased simply by eliminating the Pb source, although these levels have not returned to the values observed in rats not exposed to Pb. However, this procedure was not an effective way to resolve the changes in vascular reactivity and blood pressure induced by perinatal Pb exposure. On the basis of these reults, we evaluated the therapeutic effects of DMSA, L-arginine, and enalapril, either alone or in combination, on both Pb mobilization and cardiovascular adverse effects of perinatal Pb intoxication.

Body Pb mobilization

Independent of age, DMSA, enalapril and combination therapy, but not L-arginine, were more effective than the cessation of Pb administration in decreasing blood Pb levels. These data confirm previous reports from our laboratory demonstrating that L-arginine treatment is ineffective in decreasing blood Pb levels[24]. The mobilization of Pb from tissues can provoke an undesirable redistribution. This may explain the maintenance of high blood Pb levels after L-arginine treatment, which was shown to be capable of mobilizing Pb from tissues such as the femur, liver, and kidney[24].

Enalapril treatment was also capable of decreasing blood Pb levels to those observed in nonintoxicated rats. Perhaps this finding can be explained by an increase in Pb excretion, which results from the usual increase in the glomerular filtration rate induced by angiotensin-converting enzyme inhibitors under

conditions of low glomerular filtration, similar to that observed in cases of Pb poisoning[36]. In fact, Pb also affects kidney function; the glomerular filtration rate appears to be affected by the lowest blood Pb levels. A decreased glomerular filtration rate has been consistently observed in populations with a mean blood Pb level of $<20\ \mu g/dL$. Moreover, the increase in blood pressure and decrease in the glomerular filtration rate are closely related and probably share synergistic effects[21].

Blood pressure

Independent of age, all treatments inhibited the increase in blood pressure induced by perinatal Pb exposure.

However, in contrast to other therapies, DMSA alone was not effective in completely normalizing the blood pressure of adult rats perinatally exposed to Pb. This result corroborates previous data from our laboratory showing that 30-day DMSA treatment is unable to completely reverse the sustained increase in systolic blood pressure observed in rats exposed to Pb during adult life[24]. Contrasting reports from the literature have shown that DMSA treatment is capable of abolishing Pb-induced hypertension[16,37]. This discrepancy may be due to differences in the dosing protocols and treatment duration because these parameters are important for reversing Pb-induced hypertension[38]. Another possibility can involve differences in the mechanisms underlying the maintenance of Pb-induced hypertension that are related to different periods of animal development. This hypothesis is based on the fact that treatment with DMSA is completely effective in restoring Pb-induced hypertension in young, but not adult, rats.

L-arginine showed an increased latency in the expression of beneficial effects on blood pressure in young rats compared with that in adult rats. The opposite was observed during treatment with enalapril. Moreover, L-arginine treatment was shown to be more effective than DMSA treatment in the reversal of blood pressure in adult, but not young, rats. These observations also support the hypothesis that there are distinct mechanisms for the maintenance of Pb-induced hypertension during different periods of life.

Finally, combination therapy induced an earlier restoration of blood pressure in Pb-exposed young rats compared with the monotherapies, except for enalapril monotherapy. One possible explanation would be the additional effects of L-arginine, which is an NO precursor, DMSA, which is a chelating agent and scavenger of ROS, and enalapril, which is a renin–angiotensin system inhibitor, on blood pressure. Because the heart rate is higher in young animals than in adult animals, and because the presence of enalapril increases glomerular filtration, one can expect a higher excretion of Pb in young rats. This fact can explain the similar efficacy between enalapril and combined therapy in restoring the blood pressure of young rats exposed to Pb and the higher efficacy of combination therapy in adult rats.

Vascular reactivity

Independent of postnatal age, all treatments were able to resolve the changes in vascular reactivity induced by perinatal Pb exposure. We speculate that Pb-induced changes in vascular reactivity were caused by decreased NO and/or by a ROS and that the provision of additional NO through administration of the substrate L-arginine could act as an ROS scavenger and/or directly as a vasodilator, thus resolving the vascular changes. In addition to its chelating action, DMSA may also act as a scavenger of ROS that may contribute to the improvement in changes in vascular reactivity induced by Pb exposure[16,39]. Some other reported mechanisms by which Pb can contribute to the development of arterial hypertension are an increase in the angiotensin-converting enzyme activity and an increase in the plasma levels of renin, angiotensin II, aldosterone, and kininases[1]. In the present study, enalapril normalized the functional changes in vascular reactivity induced by perinatal Pb exposure. This result corroborates with those of previous studies showing that strategies interrupting the renin–angiotensin system also decrease cardiovascular alterations in a variety of hypertensive states, including those caused by Pb exposure[36].

Most conventional chelators are compromised by side effects, particularly their binding to essential metals, which decreases their efficacy. As a result, there is no safe and effective treatment for Pb poisoning[13,30]. Therefore, combination therapy may play a significant and important role in the abation of several toxic effects of Pb compared with monotherapies, in addition to their impressive effects in terms of blood pressure recovery. In this context, this study advances our understanding of the cardiovascular effects of perinatal Pb exposure and can aid in the development of a new therapeutic protocol for the treatment Pb-induced hypertension.

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Author contributions

Conception and design of the research, Acquisition of data, Analysis and interpretation of the data, Statistical analysis and Writing of the manuscript: Fresneda-Gaspar A, Cordellini S; Obtaining financing and Critical revision of the manuscript for intellectual content: Cordellini S.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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