Effect of L-arginine, dimercaptosuccinic acid (DMSA) and the association of L-arginine and DMSA on tissue lead mobilization and blood pressure level in plumbism

Lead (Pb)-induced hypertension is characterized by an increase in reactive oxygen species (ROS) and a decrease in nitric oxide (NO). In the present study we evaluated the effect of L-arginine (NO precursor), dimercaptosuccinic acid (DMSA, a chelating agent and ROS scavenger), and the association of L-arginine/DMSA on tissue Pb mobilization and blood pressure levels in plumbism. Tissue Pb levels and blood pressure evolution were evaluated in rats exposed to: 1) Pb (750 ppm, in drinking water, for 70 days), 2) Pb plus water for 30 more days, 3) Pb plus DMSA (50 mg kg\textsuperscript{-1} day\textsuperscript{-1}, po), L-arginine (0.6%, in drinking water), and the combination of L-arginine/DMSA for 30 more days, and 4) their respective matching controls. Pb exposure increased Pb levels in the blood, liver, femur, kidney and aorta. Pb levels in tissues decreased after cessation of Pb administration, except in the aorta. These levels did not reach those observed in nonintoxicated rats. All treatments mobilized Pb from the kidney, femur and liver. Pb mobilization from the aorta was only effective with the L-arginine/DMSA treatment. Blood Pb concentrations in Pb-treated groups were not different from those of the Pb/water group. Pb increased blood pressure starting from the 5th week. L-arginine and DMSA treatments (4th week) and the combination of L-arginine/DMSA (3rd and 4th weeks) decreased blood pressure levels of intoxicated rats. These levels did not reach those of nonintoxicated rats. Treatment with L-arginine/DMSA was more effective than the isolated treatments in mobilizing Pb from tissues and in reducing the blood pressure of intoxicated rats.

The severity of the effects of, and the extent to which the cardiovascular system is affected by, lead (Pb) appear to be influenced most directly by the Pb dose and the duration of Pb exposure. Some epidemiological data are compatible with the idea that chronic low-level Pb exposure plays a role in the development of hypertension in occupationally Pb-exposed persons (1,2) and in the general population (3,4). The increased vas-
cular responsiveness to pressor agents is one of several factors contributing to the development of Pb-induced hypertension (5).

Vascular endothelial and smooth muscle cells are major components of blood vessels. Endothelial cell involvement in plumbism was previously reported by Pirkle et al. (3), who suggested a blockade of nitric oxide (NO) generation in Pb-induced hypertension. Moreover, Pb can block NO production in cerebral endothelial cells (6), macrophages (7), and aorta endothelial cells (8). More recently it was found that Pb-induced hypertension in rats may be related to an increase in reactive oxygen species (ROS) (9-11).

The use of chelating agents, such as dimercapto-succinic acid (DMSA), is the recommended therapy in heavy metal intoxication. Besides the chelating action, DMSA also presents a scavenger action of ROS (2).

Thus, the objective of the present study was to evaluate the therapeutic potential of L-arginine (the precursor of NO) and of the combination of L-arginine and DMSA on both Pb mobilization from tissues and blood pressure levels in plumbism.

Male Wistar rats were housed five per cage and had free access to food and water, except during testing. They were exposed to a 12-h light-dark cycle and the room temperature was controlled at 25 ± 1°C. The rats were provided by the animal facilities of Universidade Estadual Paulista. Animal procedures were in accordance with the guidelines for animal care prepared by the Committee on Care and Use of Laboratory Animal Resources, National Research Council, Washington DC, USA.

Animals were first exposed to Pb at the age of 5-6 weeks, when they weighed 150-170 g. Experiments were performed on rats weighing 310-410 g.

The levels of Pb in blood, kidney, femur, liver and aorta, as well as the evolution of blood pressure were evaluated in: 1) rats exposed to Pb (750 ppm, in the form of Pb acetate in their drinking water ad libitum) for 70 days (Pb group), and rats that received water (water group) or sodium acetate (supplying an identical amount of acetate) during the same period, 2) rats exposed to Pb for 70 days and then receiving water for 30 additional days (Pb/water group), and rats that received water for 100 days (water group), and 3) rats exposed or not to Pb that later received DMSA (25 mg/kg, orally by gastric gavage, twice a day for 6 days per week) (water/DMSA or Pb/DMSA groups), L-arginine (0.6% in their drinking water ad libitum) (water/L-arginine or Pb/L-arginine groups), and the combination of L-arginine/DMSA (water/L-arginine/DMSA or Pb/L-arginine/DMSA groups) for 30 additional days.

One day after cessation of treatments, the animals were decapitated and trunk blood was collected for Pb determination. The levels of Pb in the kidney, femur, liver and aorta were determined in rats submitted to perfusion with heparinized saline (0.9%) before tissue removal. Pb content was measured using an atomic absorption spectrometer (SpectrAA 220Z-Varian).

Systolic blood pressure was determined by tail-cuff plethysmography (Narco Bi-Systems, Houston, TX, USA) in conscious rats. Three measurements of blood pressure were made, and the mean of these three measurements was recorded. Blood pressure was determined before the beginning of Pb exposure and once a week for 70 or 100 days depending on the experimental protocol. Matching controls were used.

Results were compared by the unpaired Student t-test and by analysis of variance when appropriate. P<0.05 was taken as statistically significant. The Tukey-Kramer test was used to test differences among means.

Pb exposure caused an increase in the levels of this metal in the blood, liver, femur, kidney and aorta (Table 1). The levels of Pb in tissues decreased significantly after cessation of Pb administration, except in the aorta.
L-arginine and DMSA effects in plumbism

However, these levels did not reach the values observed in rats not exposed to Pb (Table 1). The data also demonstrated that all treatments were effective in mobilizing Pb from the kidney, femur, and liver, except for the DMSA treatment, which did not mobilize Pb from the femur (Table 1). However, Pb mobilization from the aorta was only effective with the L-arginine/DMSA treatment (Table 1). Furthermore, blood Pb concentrations in Pb-treated groups were not different from those of the Pb/water group. An exception was the L-arginine/DMSA treatment that decreased the blood Pb concentration compared to the Pb/water group (Table 1).

In addition, Pb intoxication caused a significant increase in blood pressure starting on the 5th week (Figure 1). The DMSA treatment (4th week), L-arginine treatment (4th week) and the arginine/DMSA combination (3rd and 4th weeks) were effective in decreasing the blood pressure levels of rats exposed to Pb (Figure 1). However, these levels did not reach those of rats not exposed to Pb (Figure 1).

Treatment with sodium acetate did not cause any change in Pb mobilization or blood pressure levels of rats (data not shown).

L-arginine, DMSA, and the combination of L-arginine and DMSA treatments were more effective than the cessation of Pb administration in decreasing Pb levels in the liver, femur, and kidney, although the values did not reach those observed in rats not exposed to Pb. These data confirm previous reports demonstrating that DMSA treatment is more effective than the cessation of Pb administration in mobilizing Pb from tissues. However, depending on the dose and time of exposure, Pb levels may not reach those observed in rats not exposed to Pb (2,12,13).

In contrast to what was observed in the other tissues, the combination of L-arginine and DMSA was the only effective treatment

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<th>Table 1. Tissue lead (Pb) concentrations in rats.</th>
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Water: rats not exposed to lead; Pb: rats exposed to 750 ppm Pb in drinking water ad libitum for 70 days; water/DMSA, water/L-Arg and water/L-Arg/DMSA: rats not exposed to Pb that received dimercaptosuccinic acid (DMSA), L-arginine (L-Arg) and the combination of L-Arg and DMSA, respectively, for 30 days; Pb/water, Pb/DMSA, Pb/L-Arg and Pb/L-Arg/DMSA: rats exposed to Pb for 70 days that received DMSA, L-Arg and the combination of L-Arg and DMSA, respectively, for 30 additional days. Values are reported as means ± SEM (N = 4-10).

*P<0.05 compared to water.

†P<0.05 compared to Pb/water. This comparison was not possible with water/DMSA in blood.

‡P<0.05 for Pb/treatment compared to the respective water treatment. This comparison was not possible with DMSA treatment in blood.

*P<0.05 for Pb/water compared to Pb (ANOVA and Tukey-Kramer test).
in reducing the Pb levels of the aorta and of the blood of intoxicated rats. However, these levels did not reach those observed in non-intoxicated rats. The mobilization of Pb from tissues favors its redistribution. This may explain, the maintenance of high levels of Pb in blood after the DMSA and the L-arginine treatments which were capable of mobilizing Pb from tissues. The high levels of Pb in blood under these conditions may also explain the non-mobilization of Pb from the aorta. This hypothesis is reinforced by the observation that the combination of L-arginine and DMSA, which caused a significant decrease in Pb levels in blood, was capable of reducing Pb levels in the aorta. It should be pointed out that the present study is the first report in the literature showing that L-arginine is an effective agent in mobilizing Pb from tissues, including bone tissue. The literature has shown that sulfur amino acids such as methionine and cysteine, and amino acids metabolically related to them, increase the bioavailability of glutathione, which is

Figure 1. Weekly evolution of blood pressure. A, Rats exposed to 750 ppm lead in drinking water ad libitum for 70 days (Pb) and rats that later received water for the same period of time (water). B, C, D, Rats exposed or not to Pb for 70 days that later received DMSA (water/DMSA, Pb/DMSA), L-arginine (water/L-Arg, Pb/L-Arg), and the combination of L-Arg and DMSA (water/L-Arg/DMSA, Pb/L-Arg/DMSA) for 30 additional days. Values are reported as means ± SEM (N = 6). *P<0.05 for Pb/treatment compared to water; †P<0.05 for Pb/treatment compared to Pb/water (Student t-test and ANOVA followed by the Tukey-Kramer test).
useful in chelating Pb, counteracting the toxic effects of the metal and may be used as supportive therapy (14,15). Moreover, in metalloregulatory proteins, metals are often conveniently located at binding sites and bound to cysteine residues. Several lines of evidence indicate that cysteine-rich metal-binding proteins, as well as redox-sensitive metal clusters of metalloproteins, are natural sensors of bioradicals like NO (16). In addition, Misra et al. (17) showed that NO mediates cadmium release from metallothionein. Thus, an increase in NO levels by L-arginine treatment might displace Pb from its cell binding sites.

The sustained increase in systolic blood pressure observed in rats treated with Pb for 70 days confirms previous reports in the literature showing that arterial hypertension is associated with plumbism (2,3). The combination of L-arginine and DMSA induced an earlier decrease in the blood pressure of Pb-intoxicated rats compared to the isolated treatments. One possible explanation would be the additional effect of L-arginine, the NO precursor (18), and the effects of DMSA, a chelating agent and scavenger of ROS (2,19), on blood pressure. However, no treatment was effective in normalizing the blood pressure of Pb-intoxicated rats, in contrast to literature reports showing that DMSA treatment is capable of abolishing Pb-induced hypertension (2,20). This discrepancy may be due to differences in the protocols related to the doses and duration of the treatment, since it was previously reported that these parameters are important in reversing Pb-induced hypertension (12).

The present study showed 1) the action of L-arginine as a Pb chelator, and 2) an anticipation in the reversal of the blood pressure levels, as well as a larger mobilization of Pb from the aorta and blood with the combination of L-arginine and DMSA compared to the isolated treatments. These observations suggest a therapeutic potential for the combination of L-arginine and DMSA in plumbism.

Acknowledgments

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References