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Hydrogen Peroxide Reduces Lead-Induced Oxidative Stress to Mouse Brain and Liver

R. G. Li · T. T. Li · L. Hao · X. Xu · J. Na

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Abstract Lead (Pb) intoxication may initiate many disorders in human and animals. This study investigates the role of exogenous hydrogen peroxide (H_2O_2) in inducing mouse tolerance to Pb exposure. Results showed that the simultaneous application of 1.2 μg H_2O_2 per kg body weight efficiently protected mice against the Pb-caused injury, as revealed by decreased growth suppression caused by the Pb stress, increased antioxidative enzyme activity, reduced lipid peroxidation, and the protective effect on the nuclear DNA integrity. To our knowledge, this is the first finding of this sort.

Keywords Mice · Lead · Hydrogen peroxide · Oxidative stress

Lead (Pb) is one of the major environmental hazards for humans, animals and plants due to its high toxicity, wide distribution and persistence in the environment (Lockitch 1993). Lead is released into the environment by traffic, industrial processes, as a by-product of mineral fertilizers and from other sources, and then is transferred to the food chain (Nriagu and Pacyna 1988). Habitants of big cities are at risk of Pb intoxication which can cause serious occupational diseases. Several lines of evidence suggest that Pb intoxication may initiate oxidative stress possibly by overproducing reactive oxygen species (ROS), such as $O_2^{\bullet -}$ and H_2O_2, which produce extremely toxic hydroxyl radicals ($^{\bullet}OH$). These species and free radicals are subject to alterations in the functionality of membranes by inducing changes in lipid composition, causing DNA damage, inactivating enzymes and even causing cell death (Hermes-Lima et al. 1991; Bechara et al. 1993; Hartwig 1994; Adonaylo and Oteiza 1999a, b; Kanno et al. 2000). Higher organisms, however, have evolved a complex of antioxidant defense system comprising low-molecular components such as ascorbate and glutathione, and enzymatic components such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), which are involved in the detoxification of $O_2^{\bullet -}$ and H_2O_2 respectively, thereby preventing the formation of $^{\bullet}OH$ radicals (Valko et al. 2007). Until relatively recently, H_2O_2 was viewed mainly as a toxic cellular metabolite (Neill et al. 2002). Especially in animals, hydrogen peroxide has been implicated as an important mediator of cytotoxicity and genotoxicity (Hyslop et al. 1995; Lueken et al. 2004). However, it is becoming increasingly clear that H_2O_2 functions as a signaling molecule that mediates responses to various biotic and abiotic stimuli in both plant and animal cells (Neill et al. 2002). In plant tissue, the role of ROS (mainly H_2O_2) during biotic and abiotic stress has become a subject of considerable interest given that ROS have been implicated in processes leading to plant stress acclimation (for review, see Dat et al. 2000). In animals, beneficial effects of ROS have also been clearly demonstrated by a growing body of evidence. Various ROS-mediated responses actually protect the cells against ROS-induced oxidative stress and re-establish or maintain "redox homeostasis" (Droge 2002; Valko et al. 2007). However, to our knowledge, very little information is available whether H_2O_2 treatment leads to animal tolerance to Pb exposure. In this study, the potential Pb stress-protective effect of H_2O_2 was investigated using mice as an experimental model. The aim was to determine

R. G. Li and T. T. Li contributed equally to this work.

R. G. Li · T. T. Li · L. Hao (✉) · X. Xu · J. Na
Department of Biology, Shenyang Normal University, Shenyang 110034, People's Republic of China
e-mail: haolinwj2001@yahoo.com.cn

the relationship between Pb-caused cytotoxicity (as lipid peroxidation) and genotoxicity (as DNA damage) and H_2O_2-induced antioxidant activities.

Materials and Methods

Four to 5-week-old healthy male mice weighing 23 ± 2 g were randomly divided into six groups and kept to a cage (20 × 20 × 15 cm) containing five animals with food and water under a 12 h light/12 h dark cycle in humidity (60 ± 10%) and temperature (25 ± 2 °C) controlled rooms. The first group served as control, while the other five groups were administered by oral gavage of Pb^{2+} solution (300 mg kg^{-1} body weight of lead acetate) supplied with or without H_2O_2 (0.5, 1, 2, and 5 mM H_2O_2 solution corresponding to 0.6, 1.2, 2.4 and 6 μg H_2O_2 per kg body weight, respectively) every other day for 5 days. The concentration of Pb used was derived from a pre-experiment. Briefly, mice were administered by oral gavage of Pb solutions of 0, 100, 200, 300, and 400 mg kg^{-1} body weight of lead acetate, respectively. The result showed that under treatment of 300 mg kg^{-1} or above of lead acetate, all indices determined in this paper had changed significantly compared with controls. Samples (brain and liver) were collected on day 5 of Pb exposure and washed three times with ice-cold saline, weighted, wrapped with aluminum foil and stored in liquid nitrogen before use.

Tissues at 0.5 g were ground in liquid nitrogen with pestle/mortar. The tissue powder was added to a test tube containing 5.0 ml ice-cold extract solution consisting 50 mM phosphate buffer, pH 7.0, 1 mM dithiothreitol. After mixing, the solution was incubated for 15 min on ice. The homogenate was centrifuged at 12,000×g for 15 min, and the supernatant was collected to determine the following: (1) SOD (EC 1.15.1.1) activity was assayed using the photochemical nitroblue tetrazolium (NBT) method. The assay was based on SOD ability to inhibit reduction of nitroblue tetrazolium (NBT) to form formazan by superoxide as described previously (Beyer and Fridovich 1987). One unit of SOD was defined as the amount of enzyme necessary to inhibit the reaction of NBT by 50%. (2) CAT (EC 1.11.1.6) activity was determined by directly measuring the decomposition of H_2O_2 at 240 nm for 3 min as described by Aebi (1983). (3) POD (EC 1.11.1.7) activity was estimated according to Hemeda and Klein (1990), and (4) the level of lipid peroxidation was determined by measuring the level of thiobarbituric acid reactive substance (TBARS) following Esterbauer and Cheeseman (1990). The samples were mixed with 1 mL of trichloroacetic acid (TCA) 10% and 1 ml of thiobarbituric acid (TBA) 0.67% and were then heated in a boiling water bath for 15 min. TBARS were determined by the absorbance at 535 nm and expressed as nM of malondialdehyde (MDA) formed. Protein concentration was estimated by the method of Lowry et al. (1951) using bovine albumin as standard. DNA isolation from the brain and liver and agarose gel electrophoresis were performed based on the methods of Sambrook et al. (1989). Each experiment was repeated at least three times with five replications per treatment. The statistical analysis of the data was performed using one-way ANOVA procedure.

Results and Discussion

Figure 1 showed the changes of mouse body weight during Pb exposure and H_2O_2 treatment. At day five after treatment, the body weight of Pb-intoxicated mice increased 3.5, 3.6, 18, 5.5, and −4.0% in the presence of 0, 0.6, 1.2, 2.4, and 6.0 μg kg^{-1} body weight of exogenous H_2O_2, respectively, while the control animal weight increased 10%. This indicated that the growth of mice was inhibited due to Pb exposure, as well as to high concentration of H_2O_2. However, at 1.2 μg kg^{-1}, H_2O_2 not only completely eliminated the inhibition, but also significantly promoted growth. On the other hand, although no influence on the growth was detected with lower levels of H_2O_2 (for example at 0.6 μg kg^{-1}), relatively higher levels of H_2O_2 (such as 6.0 μg kg^{-1}) resulted in deterioration of growth inhibition. These data further substantiated the "two-faced" character of ROS.

Figure 2 presented the changes of activities of SOD, POD and CAT in mouse brain and liver. Compared with the control, lead significantly ($P < 0.05$) inhibited the activities of SOD, POD and CAT, which decreased by 29, 50 and 64% in brain and 35, 37 and 31% in liver, respectively (Fig. 2). However, an alleviation of the Pb-caused suppression occurred upon the treatment with different concentrations of H_2O_2. SOD activity, responsible for elimination of superoxide radicals in cells, recovered to

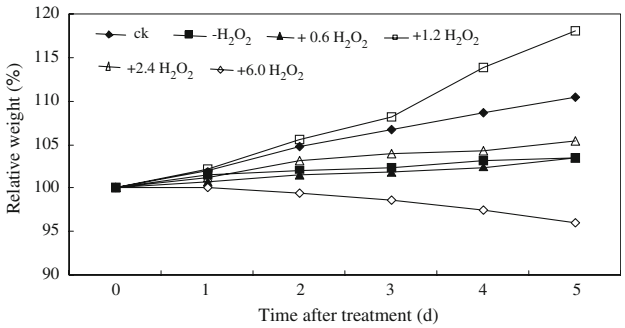

Fig. 1 Effect of H_2O_2 (μg kg^{-1}) on the body weight of mice under lead exposure

the level of control in the liver treated with all the H_2O_2 concentrations used, especially with H_2O_2 of 1.2 and 2.4 μg kg^{-1}, the activity was even higher than the control. In the brain, the situation was slightly different, where the inhibition of SOD activity was absent upon the treatment with H_2O_2 at 0.6, 1.2, and 2.4 μg kg^{-1}, but the suppression still occurred with 6.0 μg kg^{-1} H_2O_2 (Fig. 2).

The changes of POD and CAT activities in the liver were similar to the SOD, in that all concentrations of H_2O_2 treatments alleviated Pb-caused inhibition of POD and CAT. Among the concentrations of H_2O_2 tested, 1.2 μg kg^{-1} obviously induced POD, and 1.2 and 2.4 μg kg^{-1} remarkably stimulated CAT in the liver. POD activity in brain seems not susceptible to the H_2O_2 treatment, though the activity was slightly enhanced with the treatment of 1.2 μg kg^{-1} H_2O_2. CAT activity in the brain recovered with all the concentrations of H_2O_2 used, of which 1.2 μg kg^{-1} is moderate. Together, these data indicated that moderate concentrations of hydrogen peroxide alleviated Pb-caused inhibition in SOD, POD and CAT activities, which correlated well with the animal growth rate (Fig. 1).

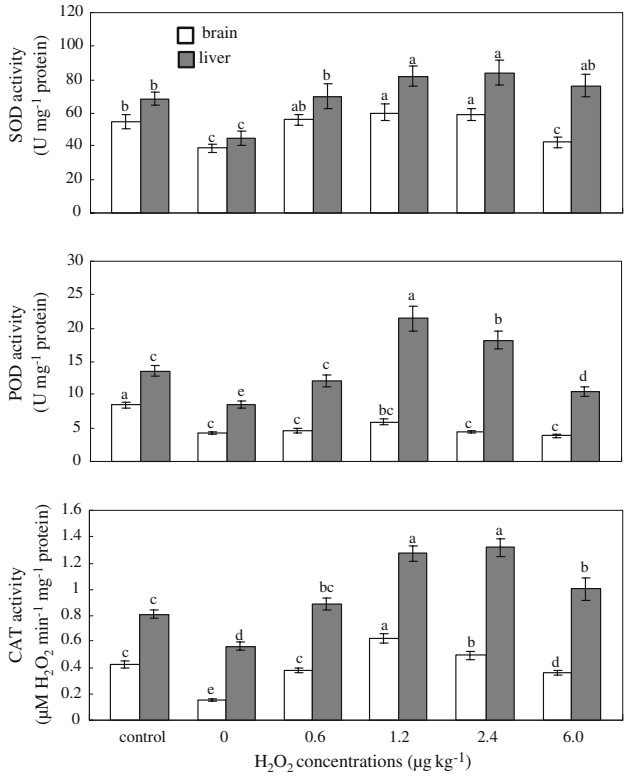

Fig. 2 Changes of activities of SOD, POD and CAT in the brain and liver treated with or without H_2O_2 under lead exposure. Each value is the mean of four individual replicates (±SE). Within each group of columns respectively represented brain and liver, different letters (*a*, *b*, etc.) indicate a significant difference at $P = 0.05$

Figure 3 demonstrated the changes of MDA content in the brain and liver. Compared with the control, Pb exposure significantly ($P < 0.01$) promoted MDA formation with an increase of about 1.4 and 0.9 times in the brain and liver, respectively. The treatment with H_2O_2 at 1.2 and 2.4 μg kg^{-1} significantly ($P < 0.01$) decreased the Pb-mediated enhancement of MDA in the brain and liver, especially with 1.2 and 2.4 μg kg^{-1}, the MDA content completely recovered to the control level. The decrease of MDA content correlated well with the increase of the antioxidant enzymes. Moreover, Fig. 3 demonstrated that the MDA level in the brain was much higher than that in the liver, which was related strongly to the antioxidant enzyme activities in which the SOD, POD and CAT in the brain were much lower than those in the liver (Fig. 2).

Figure 4 exhibited the Pb-caused nuclear DNA damage and the protective role of exogenous H_2O_2. Lead markedly caused DNA degradation in the brain and liver with a longer tails on the electrophoresis gel. All the concentrations of H_2O_2 tested alleviated the damage, of which 1.2 μg kg^{-1} strongly protected DNA from the Pb toxicity. These data further documented that moderate concentrations of exogenous H_2O_2 enhanced mouse acclimation to the Pb stress. At high concentrations, free radicals and radical-derived, nonradical reactive species are hazardous for living organisms and damage all major cellular constituents. At moderate concentrations, however, ROS play an important role as regulatory mediators in signaling processes for various biotic and abiotic stresses, as well as for other physiological functions (Droge 2002; Valko et al. 2007). Our results were in agreement with these findings. In this study, 1.2 μg kg^{-1} body weight of H_2O_2 obviously protected mice from the Pb poison in the growth rate, antioxidative level and DNA integrity (Figs. 1, 2, 3, 4). However, 6.0 μg kg^{-1} H_2O_2 produced a significant growth inhibition (Fig. 1). As for 0.6 μg kg^{-1} H_2O_2 produced no significant biological effects (Figs. 1, 2, 3, 4), the possible reasons could be due to not reaching the critical value

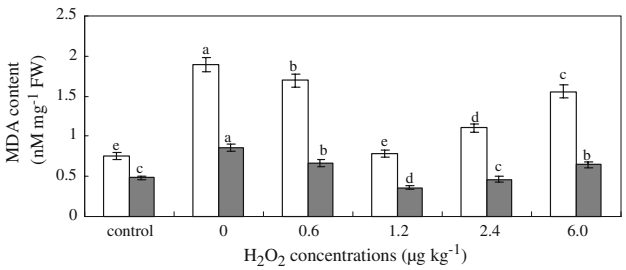

Fig. 3 TBARS levels in the brain and liver treated with or without H_2O_2 under lead exposure. Each value is the mean of four individual replicates (±SE). Within each group of columns respectively represented brain and liver, different letters (*a*, *b*, etc.) indicate a significant difference at $P = 0.05$

Fig. 4 Electrophoresis of nuclear DNA from the brain and liver treated with or without H_2O_2 under lead exposure. The identically treated samples loaded on two neighbor lanes, of which left is brain DNA, right is liver DNA. From left to right: control, treatments with 0, 0.6, 1.2, 2.4, and 6.0 μg kg^{-1} body weight of H_2O_2

needed to reestablish "redox-homeostasis". In addition, the data showed that the brain was more susceptible to the Pb-caused oxidative stress than the liver (Fig. 3), which was similar to the situation of human brain (Floyd 1999).

In conclusion, we found that moderate concentrations of hydrogen peroxide improved the symptoms of Pb poisoning in mice and protected the cellular membrane and genomic DNA from free radical-induced injury. Thus, activation of animal antioxidant enzymes by H_2O_2 plays an important role in induced tolerance to the Pb-caused oxidative stress.

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