

Korean Red Ginseng Protects Oxidative Injury Caused by Lead Poisoning

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Lead (Pb) is a metal that is generally considered to be toxic to the cardiovascular system. Pb-exposed animals display the evidence of increased oxidative stress and hypertension. The current study was designed to examine whether Korean red ginseng (KRG) has protective effects against Pb-induced hypertension and oxidative stress in Pb-exposed rats. Male Sprague-Dawley rats were randomly assigned to Pb exposure or control groups. KRG was administered in drinking water at a concentration of 100 mg/kg/day; the control group received plain drinking water. Animals in the Pb-exposed groups were provided with drinking water containing 100 ppm Pb acetate for 12 weeks. Blood pressure, plasma glutathione, blood Pb concentration, and hematologic data, such as red blood cell quantity, were determined. Pb poisoning was assessed by measuring the blood Pb concentration. Pb exposure (100 ppm) for 12 weeks resulted in a marked rise in systolic blood pressure and blood Pb concentration, as well as a significant reduction in plasma glutathione levels and red blood cell quantity. Other measurements, such as heart rate, body weight, and white blood cell quantity, were unchanged. Treatment with KRG significantly lowered blood pressure, raised plasma glutathione and increased red blood cell numbers in Pb-exposed animals; it also had no effect on heart rate, body weight, or white blood cell quantity. However, the elevated blood Pb concentration was not reduced by treatment with KRG (100 mg/kg). Taken together, these data indicate that treatment with KRG in Pb-exposed animals can reduce oxidative stress and lower blood pressure, suggesting that KRG might be protective against Pb-exposed hypertension and oxidative stress.

Keywords: Korean red ginseng, Lead, Blood pressure, Glutathione, Blood lead concentration

INTRODUCTION

Recent environmental pollution has contributed to an increase in the damage to human health caused by heavy metals. Among heavy metals, lead (Pb) has long been recognized as one of the most harmful environmental pollutants. There are many ways in which humans are exposed to Pb, through air, drinking water,

food, and contaminated soil. Increased levels of Pb in the body interfere with a variety of physiological processes, resulting in toxicity to the cardiovascular and hematopoietic systems. In particular, chronic low-level Pb exposure has been shown to cause increased blood pressure in both humans and animals [1-3]. Recent re-

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ports suggest that the inactivation of nitric oxide caused by up-regulation of reactive oxygen species generation may contribute importantly to lead-induced hypertension [4-6]. Additionally, increased nicotinamide adenine dinucleotide phosphate oxidase activity [7] and down-regulated expression of catalase [8] have also been shown to contribute to Pb-induced hypertension. Pb poisoning affects the hematopoietic system, inducing anemia by the unusual mechanism of rapid red blood cell destruction, as well as by inhibiting hemoglobin synthesis [9,10].

Korean red ginseng (KRG) has gained attention in the Orient and the West as a tonic agent, health food, and/or alternative herbal therapeutic agent. Positive pharmacological effects of KRG have been reported in the treatment of high blood pressure, atherosclerosis, and hyperlipidemia [11]. KRG has been shown to reduce oxidative damage incurred in various diseases [12]. This effect has been shown to be due to its ability to inhibit the generation of reactive oxygen species in blood vessels by reducing the activity of NADPH oxidase [13]. Furthermore, KRG can increase nitric oxide production in vascular endothelial cells [14-16], and affects vasodilation to reduce blood pressure [17]. Effects of KRG on blood pressure reduction have been shown in both humans and animals [14,15]. Administration of KRG has also been shown to reduce arterial stiffness in healthy human individuals [18].

Overall, reports suggest that KRG may be protective against hypertension, the generation of reactive oxygen species, and decreased hemopoiesis caused by exposure to Pb, but this hypothesis has not yet been confirmed. The present study aimed to examine whether KRG can have such an effect on Pb-induced physiological changes.

MATERIALS AND METHODS

Animals and Korean red ginseng treatment

Sprague-Dawley (SD) rats (average weight, 200 g) were obtained from Samtako (Osan, Korea). Extract (Hongsamjung) of KRG was provided by the Korea Ginseng Corporation (Daejeon, Korea). KRG extract (100 mg/kg/day) was administered to SD rats for 12 weeks. Pb acetate was purchased from Sigma-Aldrich (St Louis, MO, USA).

Lead exposure

Experimental animals were divided into four groups: untreated, Pb alone, Pb plus KRG, and KRG alone. Each group was housed (30×20 cm) under a 12/12-h

light/dark cycle at constant temperature (20 to 25°C) and humidity (50 to 60%). Pb exposure was achieved for 12 weeks by giving 100 ppm Pb (as 0.48 mM lead acetate) in sterilized drinking water. All animal procedures were in accordance with the Chungnam National University Guide for the Care and Use of Laboratory Animals.

Determination of lead concentration and blood cell counting

At 12 weeks after Pb exposure, rat blood (4 mL) was collected in tubes containing heparin. The blood Pb concentration was determined at the Seoul Medical Science Institute (SCL, Korea) by atomic absorption spectrometry. The number of white blood cells, red blood cells, and platelets in 3 mL of whole blood was counted at SCL.

Determination of glutathione concentration

Plasma levels of glutathione were measured using the QuantiChrom Glutathione Assay Kit DIGT-250 (Gentaur, Brussels, Belgium). Briefly, an arterial blood sample was collected in an EDTA-coated tube and centrifuged (2000 g, 5 min) to obtain plasma. Plasma (50 µL) was diluted 10-fold in PBS. Diluted plasma (240 µL) was mixed with an equal volume of assay reagent A, and centrifuged (14,000 rpm, 5 min). The mixture of supernatant (200 µL) with assay reagent B (100 µL) was incubated at RT for 25 minutes, and the level of glutathione was determined by measuring absorbance at 412 nm.

Measurement of blood pressure

Systolic blood pressure was measured in each rat's tail, initially and at 4, 8, and 12 weeks later, using a specially devised blood pressure monitoring system (ML125, AD Instruments, Colorado Springs, CO, USA). Body weights were measured using an electronic scale (MWII-6000N, CAS Co, Korea). During measurement, rats were fixed in an animal holder to prevent any moving, and pre-heated in a chamber at 35°C for 15 minutes to ensure psychological stability. A cuff with a pneumatic pulse sensor was then attached to the tail. Blood pressure values were averaged from at least three consecutive readings obtained from each rat.

Statistical analyses

Using SPSS ver. 17.0 (SPSS Inc., Chicago, IL, USA), a repeated measure ANOVA was carried out to detect differences in blood pressure among tested groups, and

one-way ANOVA was employed to compare Pb concentration, and generation of reactive oxygen species and antioxidants. A *p*-value of ≤ 0.05 was deemed to indicate statistical significance for all variables.

RESULTS AND DISCUSSION

Effect of Korean red ginseng extract on lead-induced hypertension

To determine whether KRG had a protective effect against Pb-induced hypertension, Pb alone (100 ppm) or Pb plus KRG (100 mg/kg/day) was administered to rats for 12 weeks. After every 4 weeks of treatment, the blood pressure was measured using the tail-cuff protocol described in the Materials and Methods. An increase in blood pressure was observed at 8 weeks after exposure to Pb (100 ppm) (Fig. 1). After 12 weeks of treatment, the blood pressure of the Pb plus KRG group (134.8±2.0 mmHg) was significantly lower than that of rats exposed to Pb alone (144.4±1.5 mmHg) (Fig. 1). This suggests that KRG has a suppressive effect on Pb-induced hypertension; however, no effect on heart rate or body weight was observed in the four test groups (Table 1).

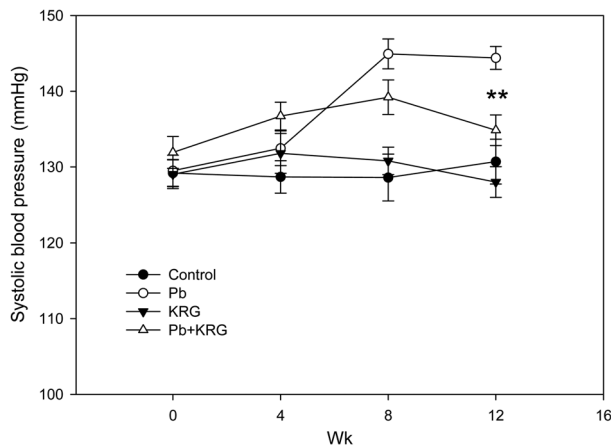


Fig. 1. Effect of Korean red ginseng (KRG) on systolic blood pressure in rats exposed to lead (Pb) acetate (100 ppm). KRG (100 mg/kg/day) was administered for 12 weeks. Each point represents the mean with standard error (*n*=14-15). Pb, Pb-treated group; KRG, KRG-treated group; Pb+KRG, Pb- and KRG-treated group. ***p*<0.01.

It is well-known that chronic Pb exposure is associated with elevated blood pressure. In multivariate-adjustment models, a blood Pb concentration ≥ 5 $\mu\text{g/dL}$ has been shown to be associated with higher systolic and diastolic blood pressure (BP) in blacks, but not whites [19]. Compared with their counterparts with a low blood Pb concentration, black men with a blood Pb concentration ≥ 5 $\mu\text{g/dL}$ had systolic and diastolic BPs that were 1.67 and 1.68 mmHg higher, respectively; black women had systolic and diastolic BPs that were 2.48 and 2.22 mmHg higher, respectively [19]. Furthermore, chronic low-dose Pb exposure has been shown to increase reactive oxygen species generation, decrease nitric oxide generation, and alter the endothelium-independent relaxation response [20]. The present study confirms that blood pressure can be increased by Pb exposure, and suggests the possibility that long-term administration of KRG (100 mg/kg/day) may interfere with pathophysiological mechanism(s) of hypertension. Previously, Kim *et al.* [13] suggested that saponin compounds in KRG may down-regulate hypertension; however, this has yet to be clarified.

Effect of KRG on lead-induced oxidative injury

To determine the effect of chronic Pb exposure on antioxidant capacity in rat blood, levels of glutathione were measured. Glutathione, a tri-peptide antioxidant, is found in all living cells, and helps protect against disease and aging. It plays an important role in oxidation-reduction in cells, and is involved in detoxification and immune enhancement. The concentration of glutathione in the plasma of untreated rats was 62.2 ± 7.9 μM ; rats treated with Pb had significantly lower levels (47.1 ± 9.0 μM) (Fig. 2). Interestingly, plasma glutathione levels in blood from rats treated with KRG alone or Pb plus KRG were comparable to those in untreated controls (Fig. 2). The results demonstrate that KRG administration alone does not change plasma glutathione, but it can antagonize the reduction of plasma glutathione caused by Pb exposure. Furthermore, we confirmed that Pb poisoning can cause oxidative injury by decreasing the plasma level of glutathione, and that

Table 1. Hemodynamic changes induced by Korean red ginseng (KRG) in the lead (Pb) exposed and control rats

	Control (<i>n</i> =10)	Pb (<i>n</i> =15)	KRG (<i>n</i> =10)	KRG+Pb (<i>n</i> =14)
Blood pressure (mmHg)	130.7±3.0	144.4±1.5*	128.0±2.0	134.8±2.0 [#]
Heart rate (beat per min)	355±12	370±11	349±13	374±10
Body weight (g)	490±9	479±11	481±9	494±8

Values are presented as mean±SE.

**p*<0.05 vs. control group, [#]*p*<0.05 vs. Pb treated group.

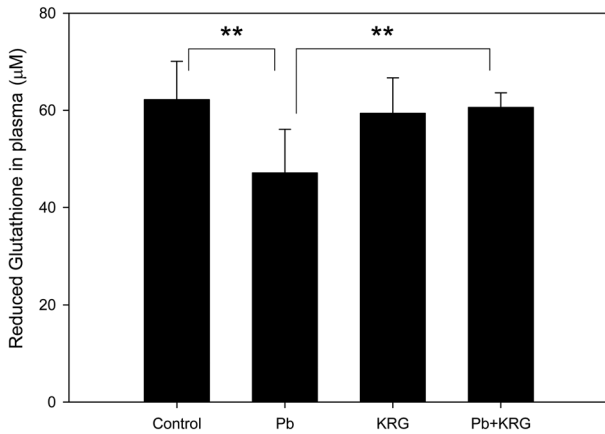


Fig. 2. Effect of Korean red ginseng (KRG) on glutathione concentrations in the plasma of rats exposed to lead (Pb) (100 ppm) and KRG extract (100 mg/kg/day) for 12 weeks. Each bar represents the mean and standard error ($n=6$). Pb, Pb-treated group; KRG, KRG-treated group; Pb+KRG, Pb- and KRG-treated group. ** $p<0.01$.

KRG enhanced the production of the reduced form of glutathione, which prevents oxidative injury.

Effect of KRG on plasma lead concentration

To determine the effect of long-term administration of KRG on Pb accumulation, plasma Pb concentrations were determined using atomic absorption spectrometry. The concentration of Pb in plasma from rats treated with KRG for 12 weeks (0.23 ± 0.05 µg/dL, $n=6$) was comparable to that of untreated control rats (0.32 ± 0.04 µg/dL, $n=6$) (Table 2). The plasma concentration of Pb in rats provided with drinking water containing 100 ppm Pb acetate for 12 weeks was 14.60 ± 0.71 µg/dL; this was about 50-fold higher than that measured in untreated control rats. As the World Health Organization and the Centers for Disease Control and Prevention define childhood Pb poisoning as a whole-blood Pb concentration ≥ 10 µg/dL [20-23], our animal model of Pb poisoning covers an appropriate range of concentrations. Treatment with KRG (100 mg/kg/day) for 12 weeks was unable to reduce the elevated blood Pb concentration (Table 2).

Table 2. Changes of Plasma lead (Pb) concentration induced by Korean red ginseng (KRG) in the lead exposed and control rats

Plasma Pb (µg/dL)	Control ($n=6$)	Pb (100 ppm) ($n=7$)
Basal (vehicle)	0.32 ± 0.04	$14.60\pm 0.71^*$
KRG (100 mg/kg)	0.23 ± 0.05	14.72 ± 0.77

Values are presented as mean \pm SE.

* $p<0.05$ vs. control group.

Effect of KRG on hematological alterations induced by long-term lead exposure

To investigate the effect of KRG on hematological alterations induced by long-term Pb exposure, the number of red blood cells, white blood cells, and platelets in the blood was determined in rats provided with drinking water containing 100 ppm Pb acetate for 12 weeks. The number of red blood cells in long-term Pb-exposed rats ($7.11\pm 0.24\times 10^6/\text{mm}^3$) was significantly lower than that of untreated controls ($7.71\pm 0.12\times 10^6/\text{mm}^3$) and KRG-treated rats ($7.58\pm 0.13\times 10^6/\text{mm}^3$) (Table 3). The number of red blood cells in Pb plus KRG-treated rats was also higher than that of rats exposed to Pb alone, but this difference was not statistically significant (Table 3). The number of white blood cells and platelets in Pb-exposed rats was significantly increased, compared with controls, but no such difference was found when comparing controls with KRG-treated rats (Table 3). Long-term KRG treatment increased the number of white blood cells ($6.49\pm 1.88\times 10^3/\text{mm}^3$), but this increase was not significantly different from controls (Table 3). Pb plus KRG treatment reduced the increase in white blood cell number ($5.33\pm 0.47\times 10^3/\text{mm}^3$) compared with Pb alone, but this also was not statistically significant (Table 3). Pb poisoning causes anemia by affecting the hematopoietic system. The present study confirmed unusual decreases in red blood cells in Pb-treated rats, which did not recover with KRG treatment. Pb seriously disrupts the function of delta-aminolevulinic acid dehydratase, which plays a role in heme synthesis [9,10]. Because KRG compounds enhance hematopoiesis [24], long-term KRG treatment may alleviate anemia by reversing Pb-induced heme synthesis disruption.

Table 3. Hematological findings induced by Korean red ginseng (KRG) in the lead (Pb) exposed and control rats

	Control ($n=10$)	Pb ($n=7$)	KRG ($n=10$)	KRG+Pb ($n=6$)
RBC ($\times 10^6/\text{mm}^3$)	7.71 ± 0.12	$7.22\pm 0.1^*$	7.58 ± 0.13	7.66 ± 0.25
WBC ($\times 10^3/\text{mm}^3$)	5.42 ± 0.62	6.49 ± 1.88	5.15 ± 0.49	5.33 ± 0.47
Platelet ($\times 10^3/\text{mm}^3$)	843 ± 16	1141 ± 205	875 ± 26	1026 ± 61

Values are presented as mean \pm SE.

RBC, red blood cell; WBC, white blood cell.

* $p<0.05$ vs. control group.

Summary

Although KRG did not affect heart rate, body weight, or white blood cell numbers, treatment with KRG significantly lowered blood pressure, raised plasma glutathione, and increased the number of red blood cells. Despite these changes, the elevated blood Pb concentration was not reduced by treatment with KRG (100 mg/kg), suggesting that KRG does not prevent Pb uptake. These data indicate that treatment with KRG in Pb-exposed groups reduced oxidative stress and lowered blood pressure. This suggests that KRG might protect against Pb-induced hypertension and oxidative stress.

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