Research Article

Korean Red Ginseng enhances cardiac hemodynamics on doxorubicin-induced toxicity in rats

Young-Jin Jang\textsuperscript{1,}*, Dongbin Lee\textsuperscript{2,}*, Mohammad Amjad Hossain\textsuperscript{1}, Adithan Aravinthan\textsuperscript{1}, Chang-Won Kang\textsuperscript{1}, Nam Soo Kim\textsuperscript{1}, Jong-Hoon Kim\textsuperscript{1,}\textsuperscript{*}

\textsuperscript{1} College of Veterinary Medicine, Biosafety Research Institute, Jeonbuk National University, Iksan-city, Republic of Korea
\textsuperscript{2} College of Veterinary Medicine, Gyeongsang National University, Jinju, Republic of Korea

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\textbf{A B S T R A C T}

\textbf{Background:} Korean Red Ginseng (KRG) has been known to possess many ginsenosides. These ginsenosides are used for curing cardiovascular problems. The present study show the protective potential of KRG against doxorubicin (DOX)-induced myocardial dysfunction, by assessing electrocardiographic, hemodynamic, and biochemical parameters and histopathological findings.

\textbf{Methods:} Animals were fed a standard chow and adjusted to their environment for 3 days before the experiments. Next, the rats were equally divided into five groups (n = 9, each group). The animals were administered with KRG (250 and 500 mg/kg) for 10 days and injected with DOX (20 mg/kg, subcutaneously, twice at a 24-h interval) on the 8th and 9th day. Electrocardiography and echocardiography were performed to study hemodynamics. Plasma levels of superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde were measured. In addition, the dose of troponin I and activity of myeloperoxidase in serum and cardiac tissue were analyzed, and the histopathological findings were evaluated using light microscopy.

\textbf{Results:} Administration of KRG at a dose of 250 and 500 mg/kg recovered electrocardiographic changes, ejection fraction, fractional shortening, left ventricular systolic pressure, the maximal rate of change in left ventricle contraction ($+\text{dP/dt}_{\text{max}}$), and left ventricle relaxation ($-\text{dP/dt}_{\text{max}}$). In addition, KRG treatment significantly normalized the oxidative stress markers in plasma, dose dependently. In addition, the values of troponin I and myeloperoxidase were ameliorated by KRG treatment, dose dependently. And, KRG treatment showed better histopathological findings when compared with the DOX control group.

\textbf{Conclusion:} These mean that KRG mitigates myocardial damage by modulating the hemodynamics, histopathological abnormality, and oxidative stress related to DOX-induced cardiomyopathy in rats. The results of the present study show protective effects of KRG on cardiac toxicity.

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1. Introduction

Doxorubicin (DOX) is a broad-spectrum, potent tumor chemotherapeutic agent widely used in the treatment of solid and hematopoietic tumors, including breast cancer and leukemia [1]. However, the clinical availability of the drug is lost because of dose-dependent cardiotoxicity [2]. There are many mechanisms to explain the cardiotoxicity induced by DOX, including free radical production. The free radical can damage cells, inducing altered intracellular calcium metabolism and mitochondrial apoptosis signaling pathways, and induce lipid peroxidation of the cell membrane [3–5]. Besides the free radical production, DOX can induce cardiotoxicity by regulating the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) [6,7] as well as malondialdehyde (MDA). In addition, DOX-induced toxicity has been demonstrated by heart dysfunction [8]. In humans, the risk of using anthracyclines such as DOX has been studied and they were found to be associated with cardiac death, which occurs most prominently due to the prolongation of the QT interval that triggered torsades de pointes.

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[9]. Through various mechanisms, the use of DOX may consequently result in refractory heart dysfunction, leading to serious cardiomyopathy [10]. Because the cardiotoxicity induced by DOX in rats offers a relevant model to evaluate these cardiac problems, we chose to study the cardioprotective effect of Korean Red Ginseng (KRG) in the present model.

KRG is made by steaming and drying Panax ginseng Meyer, suggesting chemical transformation by heat [11]. Panax ginseng Meyer is available as a traditional herbal remedy in the Orient and exhibits many valuable functional activities such as antioxidant, antiapoptotic, anti-inflammatory, and antitaging properties. Most of all, KRG is used more often for preventing cardiovascular and diabetic diseases [12,13]. Therefore, in the present study, to examine the effects of KRG—on DOX-induced cardiac toxicity in rats, we have tried to evaluate the effects of KRG on DOX-intoxicated rat hearts using biochemical, electrocardiographic, hemodynamic, microscopic, and morphometric parameters.

2. Materials and methods

2.1. Animals

Forty-five male Wistar rats weighing 250 ± 20 g were purchased from Samtako (Seoul, Korea) and used in the present study. The animals were housed at an ambient temperature of 22 ± 2 °C and humidity of 55 ± 10 °C with 12-h light/dark cycles. All in vivo studies were conducted in accordance with international guidelines for animal experiments and the principles of laboratory animal care of Jeonbuk National University (Jeonju, Korea).

2.2. Test drugs

KRG was supplied by Korea Ginseng Corporation (KGC; Daejeon, Korea). The levels of ginsenosides in KRG, assayed by the HPLC-evaporative light scattering detector method [14], were as follows: Rg1, 2.01 mg/g; Re, 1.61 mg/g; Rf, 2.58 mg/g; Rh1, 0.95 mg/g; Rb1, 8.27 mg/g; Rg2S, 1.35 mg/g; Rc, 3.90 mg/g; Rd, 1.09 mg/g; Rb2, 3.22 mg/g; Rg3S, 1.04 mg/g; and other minor ginsenosides. KRG was mixed in tap water to the dose of 250 and 500 mg/kg.

2.3. Experimental protocols

The animals were distinguished into five groups (n = 9, each group). In the normal control (N/C) group, the animals received 0.2 % (vol/vol) starch in tap water orally once daily for 10 days, and the hearts were not subjected to DOX. In the KRG-alone (500KRG) group, the animals were administered with a dose of 500 mg/kg once a day for 10 days. In the DOX control group, the animals were administered with 0.2 % (vol/vol) starch in tap water orally once daily for 7 days and injected with DOX (20 mg/kg, subcutaneous injection twice at a 24-h interval) on the 8th and 9th day. The dose of DOX was decided by modification of previous protocols [15–18]. In the 250KRG + DOX and 500KRG + DOX groups, the animals were pretreated with 250 and 500 mg/kg KRG for 10 days by gastric gavages, respectively. At the same time, on the 8th and 9th day, 20 mg/kg of DOX was injected subcutaneously twice at a 24-h interval with KRG for 10 days (Fig. 1). The KRG dose was determined after preliminary examination of various doses.

2.4. Preparation of electrocardiographic recording

Electrocardiograms (ECGs) were recorded on the epicardial surface using an ECG apparatus (Cardio; Zimence, Germany). The rats were anesthetized by inhalation of isoproterenol. In the animals, electrodes were attached under the skin at position II, 20 min after anesthesia. The ECG parameters were assessed with a speed of 50 mm/s at 1 mV/1 cm. The ECG was obtained for 5 s. The P wave, QRS complex, QT intervals, RR intervals, and ST segments were analyzed from ECG recordings in each group. Many researchers have used the ECG apparatus to evaluate cardiotoxicity induced by DOX [19–21]. If cardiac rhythm was abnormal during the calibration, the heart was discarded.

2.5. Measurement of echocardiographic parameters

At the end of the aforementioned experiments, cardiac left ventricular systolic pressure (LVSP) and left ventricular developed pressures (+dP/dtmax and −dP/dtmin) were continuously estimated using echocardiography, the 16-channel PowerLab system (ADInstruments, Oxford, UK). Ejection fraction (EF; %) was calculated as follows: (left ventricular end-diastolic volume − left ventricular end-systolic volume)/left ventricular end-diastolic volume × 100%, and fractional shortening (FS; %) was also calculated as [left ventricular end-diastolic dimension − left ventricular end-systolic dimension]/left ventricular end-diastolic dimension × 100% [22].

2.6. Blood and tissue sampling

Blood was collected from the descending aorta into anticoagulant-containing tubes. These blood-containing tubes were centrifuged at 3,000 rpm for 10 min, and the plasma was transferred to tubes for analysis of antioxidant parameters. Evidence of oxidative stress was evaluated from SOD, CAT, GSH-Px, and MDA. SOD was analyzed using the method previously described by Mishra and Fridovich [24]. CAT was calculated using the protocol described by Aebi [25]. GSH-Px was assayed using the methods described by Rotruck et al [26]. MDA, indicative of lipid peroxidation, was confirmed by spectrophotometry [27]. After the heart tissues were isolated and washed with physiological saline, they were fixed in 10 % formalin and used for histological assay [23].

2.7. Cardiac troponin I and myeloperoxidase assay

Cardiac troponin I (cTnI) activities, a specific biomarker of cardiac damage [28], were determined using commercial kits with an ACS:180 chemiluminescence system supplied from Bayer Diagnostics (Cedex, France) [29]. And, to quantify neutrophil infiltration, the activity of myeloperoxidase (MPO), an abundant enzyme that exists in neutrophils, was analyzed using a modified method [30]. Briefly, cardiac tissue was homogenized in 50-mM K2HPO4 solution (pH 6) containing 0.5 % hexadecyltrimethylammonium bromide. The samples were centrifuged at 11,000 g for 30 min at 4 °C, and the remaining supernatants were assayed spectrophotometrically at 460 nm for the MPO assay. The activity of MPO is presented as U/mg of tissue.
2.8. Histological evaluation

Immediately after isolation of cardiac tissue, the sample was fixed in 10% neutral formalin. The tissue was embedded in paraffin block. A paraffin block of 3-mm thickness was prepared and stained with hematoxylin and eosin reagent. The sections were then viewed under an Olympus CX-40 light microscope (Olympus, Japan) for histopathological evaluation. Histological findings were divided as follows: 0, normal condition; I, minimal infiltration of inflammatory cells; II, widespread infiltration of inflammatory cells without any evidence of tissue fibrosis; and III, widespread inflammatory cell infiltration with tissue fibrosis [31]. For evaluation, the pathologist performing pathological analysis was blinded to the treatment of each group.

2.9. Statistical analysis

All statistics for data were estimated using SigmaPlot version 12.0 (Systat Software, Inc., IL, USA). Data were analyzed using one-way analysis of variance, and statistical significance was considered at P < 0.05.

3. Results

3.1. Effects of KRG on oxidative stress

The levels of the antioxidant enzymes, such as SOD, CAT, and GSH-Px, and lipid peroxidation marker, MDA, in the normal and study groups are listed in Table 1. Activities of SOD, CAT, and GSH-Px were significantly (P < 0.01) decreased in the cardiac tissue of the DOX control group, as compared with the N/C group. However, KRG treatment significantly (P < 0.05 or P < 0.01) increased the levels of SOD, CAT, and GSH-Px as compared with the DOX-alone group. Only the animals receiving 500 mg/kg KRG alone did not show any significant change when compared with the N/C group, indicating that KRG does not per se have any side effects. However, the DOX-treated group showed significantly increased levels of MDA, the end product of lipid peroxidation and a marker for oxidative stress, in cardiac tissues as compared with the N/C group (0.01). But, KRG treatment significantly decreased the MDA level, dose dependently (Table 1).

3.2. Effects of KRG on electrocardiographic indices

The representative ECG parameters of the normal and study groups are shown in Fig. 2 (top left). The animals of the N/C and KRG-alone groups showed normal ECG patterns, whereas the DOX-treated animals showed a significant decrease in the P wave and QRS complex, indicative of cardiac dysfunction [32], but the QT and RR intervals and ST segment were increased compared with those in the N/C group. However, the magnitude of P waves and QRS complexes was significantly increased by treatment of KRG, dose dependently (P < 0.05 and **P < 0.01, Fig. 2A and B). KRG also showed a significant normalization in the QT and RR intervals and ST segment (Fig. 2C, D and 2E).

3.3. Effects of KRG on hemodynamic functions

In all groups, cardiac hemodynamic parameters such as EF, FS, LVSP, +dP/dt_{max}, and -dP/dt_{max}, as the indices of contraction and relaxation, were measured [33]. To obtain the value of all parameters, the average of at least three evaluations from three different cycles in an image was obtained (Fig. 3A). The values of LVSP, +dP/dt_{max}, and -dP/dt_{max} were significantly decreased by DOX treatment, whereas 250 and 500 mg/kg KRG increased LVSP, +dP/dt_{max}, and -dP/dt_{max} (P < 0.05 and **P < 0.01) (Fig. 3B, C, and D, respectively). Similarly, decreased values of EF and FS, as the indices of cardiac contraction and relaxation, were also shown in the DOX control group (Fig. 4A and B). However, EF and FS between the N/C and 500 mg/kg KRG-alone groups were not significantly different [98.76 ± 2.95% in EF vs. 102.96 ± 2.18 in FS (N/C designated as 100%)]. These results indicate that 500 mg/kg KRG is not a significant influence in values of EF and FS. In comparison with N/C (N/C as 100%), the DOX treatment group had an average EF of 65.74 ± 6.54%. But, the EF values were 76.43 ± 5.76% for 250 mg/kg KRG and 82.62 ± 4.57% for 500 mg/kg KRG, as shown in Fig. 4A and B. These results indicate that KRG treatment significantly increases the cardiac EF dose dependently. In addition, in estimation of FS parameter, the DOX control group had an average FS of 65.43 ± 5.645%. However, as shown in Fig. 4, the FS values were 74.67 ± 7.05% and 81.54 ± 4.99% for 250 and 500 mg/kg KRG, respectively (P < 0.05 and **P < 0.01, Fig. 4A and B). These functional data were significantly ameliorated compared with those of the KRG control group. These results indicate that pretreatment with KRG is effective for protecting against the hemodynamic dysfunction by DOX in a dose-dependent manner.

3.4. Effects of KRG on cTnI and MPO activity

The cTnI activity of the normal and study groups is shown in Fig. 5A. Regarding cTnI activity, the 500 mg/kg KRG control group did not show any significant change compared with the N/C group, showing that KRG per se does not show any side effects (Fig. 5A). But, the cTnI levels in the DOX control group were significantly increased compared with those in N/C groups, namely, the N/C and

| Table 1 | Effect of KRG on plasma antioxidant parameters in myocardial infarction. |
|---------|-----------------------------|
| Group   | Superoxide dismutase (U/mg) | Catalase (U/mg) | Glutathione peroxidase (U/mg) | Malondialdehyde (nmol/mg) |
| N/C     | 1675.43 ± 83.83             | 22.78 ± 2.25   | 176.43 ± 9.81             | 5.78 ± 1.02 |
| KRG     | 1593.78 ± 92.46             | 25.92 ± 2.71   | 183.72 ± 12.63            | 6.43 ± 1.07 |
| DOX     | 965.32 ± 85.20              | 14.76 ± 2.37   | 95.73 ± 20.27             | 19.76 ± 3.25 |
| 250 KRG + DOX | 1254.28 ± 97.31          | 18.42 ± 3.76   | 132.53 ± 24.39            | 14.46 ± 2.35 |
| 500 KRG + DOX | 1299.46 ± 85.38             | 20.46 ± 1.87   | 139.56 ± 13.72            | 13.64 ± 1.97 |

Units are expressed as U/mg protein for superoxide dismutase, catalase, and glutathione peroxidase and nmol/mg protein for malondialdehyde.

a Significantly not different (P > 0.05) from N/C.

b Significantly different (P < 0.01) from N/C.

c Significantly different (P < 0.05) from DOX.

d Significantly different (P < 0.01) from DOX.
Fig. 2. The effect of KRG on electrocardiographic evaluations. The effect of KRG on (A) P wave, (B) QRS complex, (C) QT intervals, (D) RR intervals, and (E) ST segment is shown. In each group, ECG was recorded from limb lead II with a recorder. Data are presented as means ± SD (*P < 0.05, **P < 0.05 vs DOX control). DOX, doxorubicin; ECG, electrocardiogram; KRG, Korean Red Ginseng; N/C, normal control; SD, standard deviation.

Fig. 3. The effect of KRG on echocardiographic evaluations. (A) ⊗ Echocardiography was performed for rat hearts, showing the normal cardiac function such as left ventricular systolic pressure (LVSP), left ventricular contraction (+dP/dt max), and the maximal rate of change in left ventricular relaxation (−dP/dt min). (⊗) Echocardiography showed normal cardiac function, for the group treated with 500 mg/kg KRG only for 10 days. (★★) The DOX control group, injected intraperitoneally twice on the 8th and 9th day, exhibited worsening cardiac function. (⊙ and ⊘) Echocardiography was performed for groups treated with 250 and 500 mg/kg KRG for 10 days. And, quantitative values of (B) LVSP, (C) +dP/dt max, and (D) −dP/dt min are expressed. Data are presented as mean ± SD (*P < 0.05, **P < 0.01 vs DOX control). DOX, doxorubicin; KRG, Korean Red Ginseng; N/C, normal control; SD, standard deviation.
KRG control groups showed the values of 0.422 ± 0.13 U/mg and 0.35 ± 0.153 U/mg, respectively. But, for the DOX control group, a significant increase was detected (3.62 ± 0.42 mg/l), and significant decreases were observed in 250 and 500 mg/kg KRG (2.92 ± 0.35 mg/l for 250 mg/kg KRG and 2.64 ± 0.25 mg/l for 500 mg/kg KRG, *P < 0.05 and **P < 0.01, Fig. 5A). Meanwhile, in an assay of neutrophil infiltration, the MPO activity in the DOX control group was significantly increased (16.43 ± 2.22 mg/l) compared with that in the N/C (5.84 ± 0.27 mg/l) and KRG control (5.91 ± 0.32 mg/l) groups (Fig. 5B). However, KRG treatment significantly decreased the myocardial MPO activity as compared with the DOX control group, namely, the treatment of 250 and 500 mg/kg KRG led to a significant decrease (11.84 ± 1.94 U/mg of tissue for 250 mg/kg KRG and 9.76 ± 1.22 U/mg for 500 mg/kg KRG) in the elevated MPO activity (*P < 0.05 and **P < 0.01, Fig. 5B).

3.5. Effects of KRG on DOX-induced histopathological alterations in cardiac tissue

Tissue sections of the N/C and KRG groups showed normal cardiac histopathologic findings (Fig. 6A@ and ⊗). In the DOX control group, interstitial edema, hemorrhage, and severe loss of myofibrils with fiber disorganization were observed, and in higher magnifications, myofibril injury with cytoplasmic vacuolization was seen (Fig. 6A©). However, in the KRG groups, general architecture of cardiac tissue was preserved better, and myofibril loss was reduced compared with that of the DOX control group, showing reduced edema and cardiac fiber disorganization in only some regions (Fig. 6A© and ⊗). These results are summarized in Fig. 6B.

4. Discussion

In the present study, the influence of KRG was examined against DOX-induced cardiac dysfunction in rat hearts, and the cardioprotective effects were evaluated. In the previous report, we suggested that ginsenoside total saponin has cardioprotective effect against ischemia–reperfusion injury in the rodent [34], but to the best of our knowledge, there has not been a study on effect of KRG on DOX-induced cardiac toxicity. Therefore, this manuscript is of high significance in that various antitumor agents in modern medicine are used to a great extent. The significance of the present study is that some important implications about the use of KRG have been identified. First, KRG normalized ECG parameters such as P wave, QRS complex, QT/RR intervals, and ST segments in a dose-dependent manner. However, in the present study, we are unable to find the significant difference of ECG parameters at the dose of 50
and 100 mg/kg KRG (data not shown). The reason for no difference in these parameters may be attributed to the fact that the dose of 50 and 100 mg/kg did not influence the subcutaneous injection of 20 mg/kg of DOX (twice daily). If the dosage of DOX was low, treatment with 50 and 100 mg/kg KRG may be worthwhile in producing cardioprotective effect. That could be considerably important to the interrelationship between the agents. Second, KRG inhibited cardiac dysfunction as proved by the increment of −dP/dtmax as well as +dP/dtmax. And, we found that DOX induce changes in LVSP, EF, and FS. These changes may be due to cardiac toxicity induced by DOX because the EF and FS indicate the ventricular function [35]. Therefore, we suggest that DOX can improve the cardiac dysfunction because these parameters (EF and FS) may have very important meaning clinically [36]. In this regard, treatment with KRG decreased the occurrence of pathological cardiac function, suggesting the cardioprotective activity by KRG. To sum up, the increase of cardiac function may be due to the preconditioning-like action by KRG. Third, cardiac dysfunction occurs due to varied processes, leading to the increment of reactive oxygen species, which results in severe cell injury [37] and finally leads to tissue death [38]. Generally, it is reported that free radicals are eliminated by cellular antioxidant mechanisms [37]. The present results showed that the level of MDA, an index of cellular damage by free radicals, was found to be decreased in KRG-treated groups. In addition, the level of GSH-Px, indicated as a defense system against free radicals, was found to be increased in KRG-treated groups, dose dependently, indicating that KRG decreases the occurrence of free radicals. And, decreased levels of cTnI and MPO activity in KRG-treated groups suggested that there is lower cardiac dysfunction induced by KRG. These results are consistent with previous reports [10,13]. Taken together, it can be recommended that through antioxidative roles, KRG preferentially protects cardiac tissue and consequentially prevents heart dysfunction.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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