Fatty Acids Ameliorate Doxorubicin-Induced Intracellular Ca^{2+} Increase and Apoptosis in Rat Cardiomyocytes

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Doxorubicin (Dox) is a highly effective anticancer drug but exhibits cumulative dose-dependent cardiomyopathy. In this study, we investigated effects of *Magnolia* seed extract (MagS) on the Dox-induced cardiotoxicity. The results showed that MagS significantly reduces doxorubicin (Dox)-induced increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), generation of reactive oxygen species (ROS), and apoptosis in rat cardiomyocytes. Analyses of the bioactive compounds in MagS by thin layer chromatography and gas chromatography/mass spectroscopy revealed that bioactive compounds in MagS are linoleic acid, oleic acid, and palmitic acid. All three fatty acids were able to inhibit the Dox-induced increase in $[Ca^{2+}]_i$, ROS generation, and apoptosis with a similar potency. Efficacy of MagS was examined in *in vivo* using a murine Dox-induced cardiomyopathy model. Dox (12 mg/kg, intravenously) was administered to mice and treated with the MagS (2 mg/kg/d, intraperitoneally) or saline for three weeks. Dox-treated mice showed structural disarray in heart tissue, including lymphocyte infiltration and loss of body weight. In contrast, treatment of the MagS substantially attenuated the Dox-induced cardiac damages including the loss of body weight. These results indicate that fatty acids in MagS and other seeds may ameliorate cardiotoxicity of the anticancer drug.

Key words doxorubicin; fatty acid; cardiotoxicity; Ca^{2+} signaling; apoptosis; *Magnolia*

Doxorubicin (Dox) is a highly effective anticancer drug that is widely used in treatment of a broad spectrum of cancers.[2)] However, Dox exhibits serious side-effects such as manifestation of arterial and ventricular arrhythmia and development of cumulative dose-dependent cardiomyopathy.[2,3)] These Dox-induced cardiotoxicities are known to be caused mainly by ROS generation, resulting in elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) through release from sarcoplasmic reticulum.[4)] Although the cardiotoxicity of Dox is well recognized, preventive drugs have not been discovered.

Various parts of plants have been used as a traditional medicine in Chinese, Korean, and Japanese for the treatment of various diseases. *Magnolia officinalis* REHD *et* WILS. (Horpu in Chinese) has been known to treat cold-stroke, cold damage, headache, and blood impediment.[5)] Honokiol and magnolol are the known bioactive constituents in the stem barks of *Magnolia obovata* and *M. officinalis*.[6,7)] Honokiol inhibits angiogenesis *in vitro* and tumor growth *in vivo*,[8)] lipid peroxidation in rat heart mitochondria, and arrhythmia in coronary ligated rats.[9)] Magnolol is also reported to reduce myocardial ischemia/reperfusion injury in rat *via* the inhibition of neutrophils migration.[10)] These bioactive small molecules are isolated from stem bark and/or root. However, none of the study has reported the presence of bioactive substances in *Magnolia* seed.

In an effort for the reduction of Dox-induced cardiotoxicity, we evaluated ethanol extract of *Magnolia* seed. Three fatty acids were identified and effectively reduced Dox-induced cardiotoxicity in *in vitro* and *in vivo*.

MATERIALS AND METHODS

Extraction of Plant Material Dried *Magnolia* seeds (300 g) were grinded using a food processor and extracted with 1 l of ethanol for overnight at room temperature. The solvent of *Magnolia* seed extract (MagS) was evaporated using a rotary evaporator at a maximum temperature of 45 °C. The amount of dried MagS was 62 g. The MagS was dissolved in ethanol (100 mg/ml).

Isolation of Compounds The MagS was separated by thin-layer chromatography (TLC) on silica gel GF254 using the mobile phase, chloroform : *n*-hexane : 2-propanol = 30 : 50 : 20. Ten bands from top to bottom, which were visualized under visible and UV lights, were scraped. Absorbed substances were extracted with absolute ethanol, and the extracts were obtained by centrifugation and dried using SpeedVac (AES1010, Savant, NY, U.S.A.) without heating. The dried compounds were weighed and dissolved in a small volume of absolute ethanol and kept at −20 °C until use.

GC/MS Analysis Experiments were carried out on a HP-6890 GC system connected to a HP-5973 mass selective detector fitted with a DB-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film; SGE). Conditions were as follows: inlet pressure, 77.1 kPa; 8.7 ml He min^{-1}; injection volume, 1 μl; transfer line, 250 °C; electron energy, 70 eV. The GC was programmed as follows: 5 min at 70 °C, increasing at 10 °C min^{-1} to 320 °C, operated in splitless mode (60-s valve time); He carrier gas at 1 ml min^{-1}. Identification of compounds was performed by comparison of mass spectra to the *Wiley-6 Library* and the *Essential Oils Library* (*Massfinder*), by comparison with synthetic standards or retention-index data from the literature.[11—14)] All composi-

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tions were expressed as percentage (%) by area of total fatty acids.

Preparation of Rat Cardiomyocytes Cardiomyocytes were isolated from Sprague–Dawley rats, weighing 150—200 g, by the method as described previously.[15] Briefly, hearts were rapidly excised, cannulated, and subjected to retrograde perfusion on a Langendorff apparatus at 37 °C with Ca^{2+}-free Krebs–Hanseleit (KH) buffer (10 mM HEPES, 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM pyruvate, 11 mM dextrose, and 1 mM $CaCl_2$, pH 7.3) for 5 min and then with KH buffer containing 10 mM of 2.3-butanedione monoxime (BDM), 5 mM taurine, 0.075% collagenase Type II, and 0.08 mg/ml protease Type IV and X for 7 min, and washed with KH buffer containing 0.2 mM Ca^{2+}. The left ventricle was removed, chopped into small pieces, and incubated in a glass conical flask at 37 °C for 10 min with shaking. The undigested tissue was removed through a 200 μm mesh nylon filter. The Ca^{2+} concentration in the cell preparation was gradually increased up to 1 mM. Isolated myocytes were pelleted by centrifugation at 60×***g*** for 2 min at room temperature and resuspended in a stabilizing buffer (pH 7.4) containing 20 mM HEPES, 137 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 15 mM glucose, and 10 mM BDM. The cell preparation was kept in the stabilizing buffer containing 1% bovine serum albumin at room temperature about 1.5 h and then was washed three times with minimum essential medium (MEM) at 37 °C. Cardiomyocytes were seeded on the confocal dish coated with collagen (50 μg/ml) and incubated in the humidified 5% CO_2 incubator for 4 h.

Measurement of $[Ca^{2+}]_i$ $[Ca^{2+}]_i$ in quiescent cardiomyocytes was measured as described previously.[15] Briefly, isolated cardiomyocytes attached on confocal dishes coated with collagen (50 μg/ml) was loaded with Ca^{2+} indicator Fluo 3-AM (3 μM) at 37 °C for 30 min. Excess extracellular dye was removed by washing three times with KH buffer, and an additional 30 min was allowed to hydrolyze Fluo 3-AM. Changes in $[Ca^{2+}]_i$ in cardiomyocytes were determined at 488 nm excitation/530 nm emission by air-cooled argon laser system. The emitted fluorescence at 530 nm was collected using photomultiplier. One image every 6 s was scanned using confocal microscope (Nikon, Japan). For the calculation of $[Ca^{2+}]_i$, the method was used with following equation: $[Ca^{2+}]_i = K_d(F-F_{min})/(F_{max}-F)$, where K_d is 450 nM for Fluo-3 and F is the observed fluorescence levels.[16] Each tracing was calibrated for the maximal intensity (F_{max}) by addition of ionomycin (8 μM) and for the minimal intensity (F_{min}) by addition of EGTA 50 mM at the end of each measurement. During experiments, the temperature of dishes was maintained at 37 °C using a microwarm plate. Cells were pretreated with indicated dose of MagS, each fraction or fatty acid dissolved in ethanol (10 mg/ml) that was mixed with MEM prior to addition to the cells and then incubated in a humidified 5% CO_2 incubator for 20 min. Ethanol diluted in the same way was used as a control throughout the study.

Determination of Reactive Oxygen Species (ROS) Generation Measurement of hydrogen peroxide formation was performed using a fluorescent probe in isolated adult rat cardiomyocytes as previously described.[17] Briefly, equal number of cardiomyocytes attached to collagen-coated plates were loaded with 0.2 μM (final concentration) of the peroxide-sensitive fluorescent dye 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA, Molecular Probe, Eugene, OR, U.S.A.) in HEPES buffer (pH 7.4) containing 20 mM HEPES, 137 mM NaCl, 4.9 mM KCl, 1.2 mM $MgSO_4$, 15 mM glucose, and 10 mM BDM at 37 °C for 30 min, and the plates were washed two times with HEPES buffer and used for the treatment with various reagents. Indicated drugs were preincubated at 37 °C for 20 min prior to treatment with Dox (3 μM). Fluorescence of ROS in cardiomyocytes was measured every 5 min for 90 min at 488 nm exitation and 525 nm emission using a fluorescence photometer, Spectra Max Gemini (Molecular Devices, Sunnyvale, CA, U.S.A.).

Measurement of Caspase-3 Activity Enzyme activity was measured using fluorogenic substrate, *N*-acetyl Asp-Glu-Val-Asp-7-amino-methylcoumarin (Ac-DEVD-AMC) using a method provided by the manufacturer (BD Biosciences). Cardiomyocytes were attached to collagen-coated plates at 37 °C for 10 min. After removing unattached cells, the cells were further incubated at 37 °C for 2 h in M199 medium (Gibco, Invitrogen, Grand Island, NY, U.S.A.) supplemented with 2 mg/ml BSA, 2 mM L-carnitine, 5 mM creatine, 5 mM taurine, 2 mM pyruvate, 100 IU/ml penicillin, and 100 μg/ml streptomycin. For the experiments, the cells were pretreated with various indicated drugs at 37 °C for 20 min prior to addition of Dox (3 μM). At the indicated time point, the cells were washed with ice-cold PBS (140 mM NaCl, 2.7 mM KCl, and 10 mM KH_2PO_4/K_2HPO_4, pH 7.5). Excess of PBS was removed by aspiration, and the cells were immediately frozen at −80 °C after addition of 500 μl of a lysis buffer (10 mM Tris–HCl, 10 mM KH_2PO_4/K_2HPO_4, pH 7.5, 130 mM NaCl, 1% Triton X-100, and 10 mM sodium pyrophosphate). The cells were harvested, homogenized, and centrifuged at 15000 rpm at 4 °C for 30 min. Supernatants were collected, and protein concentrations were determined as described above. For *in vivo* experiment, hearts of Dox or drugs treated mice were rapidly frozen in liquid nitrogen. The tissues were homogenized with a grinder in cold lysis buffer (10 mM Tris–HCl, pH 8.0, 0.32% sucrose, 5 mM EDTA, 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 2 mM DTT) and solubilized with lysis buffer containing 1% Triton X-100 at 4 °C for 1 h. After centrifugation, supernatant was collected and protein concentration was determined with a Bradford protein assay system (Bio-Rad). Samples (100 μg protein) were incubated with the substrate (20 μM, final concentration) at 37 °C for 1 h. Fluorescence of 7-amino-4-methylcoumarin liberated from the fluorogenic substrate was determined at 380 nm excitation and 460 nm emission using a fluorescence photometer, Spectra Max Gemini (Molecular Devices, Sunnyvale, CA, U.S.A.).

Animal Experiment Female BALB/c mice, 18 to 20 g weights, 6 to 8 weeks old, were obtained from the Orient Bio Inc. (SungNam, Republic of Korea), were acclimated to the facility for 5 d and fed and *ad libitum* with regular chow. Animal Research Committee of Chonbuk National University approved the animal study in accordance with the guidelines of the National Institutes of Health (NIH publication #85-23, 1985). Mice were divided into 4 groups. Group 1 received saline only and served: Control. Group 2 received an equivalent volume of saline 1 d after the administration of Dox (12 mg/kg, IV): Dox only. Group 3 received MagS (2 mg/kg, IP) 1 d after administration of Dox (12 mg/kg. IV): Dox+

MagS. Group 4 received MagS (2 mg/kg, IP) 1 d after administration of an equivalent volume of saline: MagS only. Mouse was maintained for 3 weeks and sacrificed for analysis.

Histology Mice were anesthetized with diethyl ether and a chest region was incised. Hearts were then removed from the mice and fixed overnight in a cold 10% formalin solution. Fixed tissues were processed routinely for paraffin embedding, and 5 μm sections were used for hematoxylin-eosine staining. Stained morphology was analyzed using a microscope (Leica, Wetzlar, Germany). The extent of lymphocyte accumulation was expressed on a semiquantitative rating scale ranging from 0 (no accumulation of lymphocyte) to 3 (lymphocyte accumulation present at least of 5 sites).

Statistical Analysis Data are expressed as mean± S.E.M. Statistical comparisons were performed using one-way ANOVA. Significant differences between groups were determined using the unpaired Student's *t* test. Statistical significance was set at $p<0.05$.

RESULTS

MagS Inhibits Dox-Induced $[Ca^{2+}]_i$ Increase in Rat Cardiomyocytes As previously reported,[7] treatment of rat cardiomyocytes with Dox slowly increased $[Ca^{2+}]_i$, beginning to increase at approximately 60—70 min after Dox treatment and reached plateau at approximately 90 min and sustained (Fig. 1A). Pretreatment of MagS inhibited Dox-induced Ca^{2+} rise in a dose-dependent manner and the maximum inhibitory effect was observed with at 0.05 mg/ml (w/v) MagS (Fig. 1B). While MagS completely inhibited the Dox-induced Ca^{2+} increase at 0.05 mg/ml (Fig. 1C), this concentration of MagS alone did not show any effect on $[Ca^{2+}]_i$ (Fig. 1D). Changes in $[Ca^{2+}]_i$ are summarized in Fig. 1E.

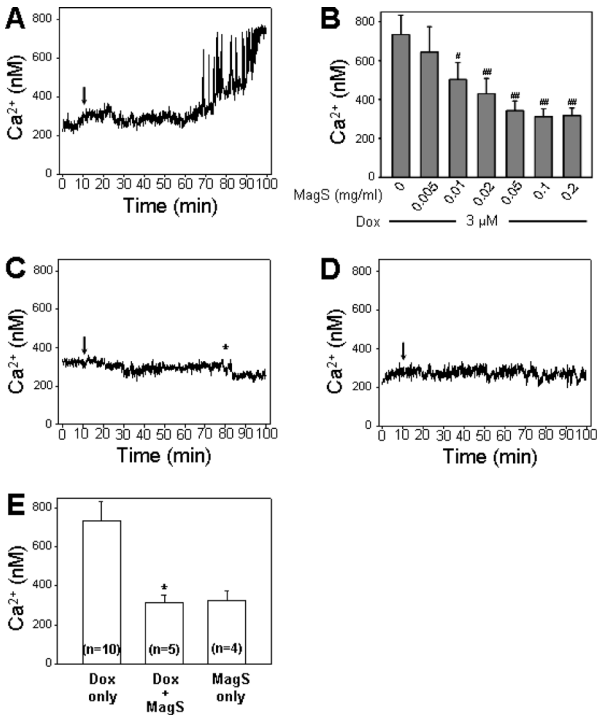

Fig. 1. Dox-Mediated Increase of $[Ca^{2+}]_i$ Is Inhibited by the MagS

Prior to addition of Dox (3 μM), rat cardiomyocytes were preincubated with and without MagS (0.05 mg/ml) as described in Materials and Methods. (A) Dox induces $[Ca^{2+}]_i$ increase without MagS, (B) dose dependent effect of MagS on Dox-induced $[Ca^{2+}]_i$ increase, (C) inhibition of Dox-induced $[Ca^{2+}]_i$ increase by MagS, (D) MagS only without Dox. (E) A direct comparison of the Ca^{2+} level at 80 min. Data are presented as mean±S.E.M. from five independent experiments. $*p<0.01$, $\#p<0.05$ and $\#\#p<0.01$ *versus* Dox.

MagS Inhibits Dox-Induced ROS Generation and Caspase-3 Activation Dox is known to induce ROS generation and apoptosis.[2,4,18] Consistent with previous observations, treatment of cardiomyocytes with Dox elevated ROS generation that was inhibited by pretreatment with MagS (0.05 mg/ml) (Fig. 2A). MagS also inhibited Dox-induced caspase-3 activation (Fig. 2B). MagS alone increased neither ROS level nor caspase-3 activity.

Screening of Bioactive Compounds Bioactive compounds in MagS were fractionated using preparative thin-layer chromatography. Visualized ten bands under visible and UV lights were scraped from thin-layer sheet. Acquired compounds were extracted with ethanol and then tested the ability of the compounds to inhibit the Dox-induced Ca^{2+} increase. As shown in Fig. 3, the third fraction contained the bioactive components. Ability of the compounds in each fraction (0.01 mg/ml) to inhibit Dox-induced Ca^{2+} raise observed at 90 min is summarized in Fig. 3M.

The third fraction that reduces Dox-induced $[Ca^{2+}]_i$ elevation in cardiomyocytes was analyzed by GC/MS methods to identify the molecules. Two major peaks were observed (Fig. 4A). The first peak with retention time of 17.291 min was *n*-hexadecanoic acid (15.708% as area %), a class of palmitic acid (Fig. 4B). The second peak overlapped with two components and the peak with retention time of 18.931 min was 9,12-octadecadienoic acid (39.684% as area %), a class of linoleic acid (Fig. 4C), and the other one (retention time, 18.971 min) was 9-octadecenoic acid (31.208% as area %), a class of oleic acid (Fig. 4D).

Fatty Acids Prevent Dox-Induced Cardiotoxicity The fatty acids obtained by GC/MS analysis were examined

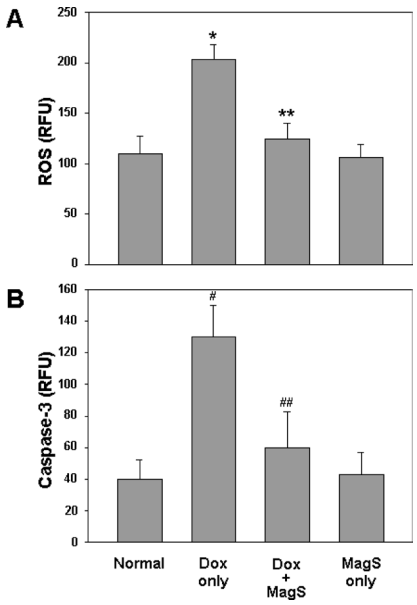

Fig. 2. Treatment of MagS Inhibited Dox-Induced ROS Generation and Caspase-3 Activation

Dox-stimulated ROS generation (A) and caspase-3 activation (B) is inhibited by treatment with MagS (0.05 mg/ml). These data are presented mean±S.E.M. from three independent experiments in duplicate. $*p<0.01$ and $**p<0.05$; $\#p<0.01$ and $\#\#p<0.05$ *versus* basal control (no drug treatment).

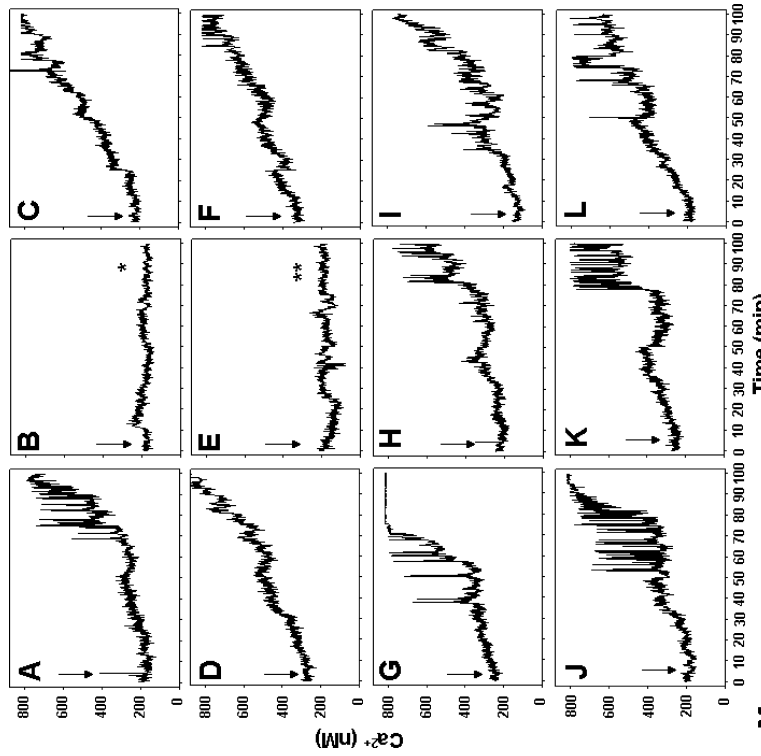

Fig. 3. Comparison of Inhibitory Effect against Dox-Mediated Increase of $[Ca^{2+}]_i$ by Fractionated MagS

Rat cardiomyocyte was preincubated with MagS (0.05 mg/ml) or each fraction of MagS (0.01 mg/ml) at 37 °C for 20 min, and then Ca^{2+} response was observed in the presence of 3 μM Dox. (A) Dox only, (B) Dox+MagS, (C to L panel) Dox+fraction no. 1 to 10, (M) a direct comparison of the Ca^{2+} level at 80 min. Data are presented as mean±S.E.M. from three independent experiments. * $p<0.01$, ** $p<0.05$ *versus* Dox.

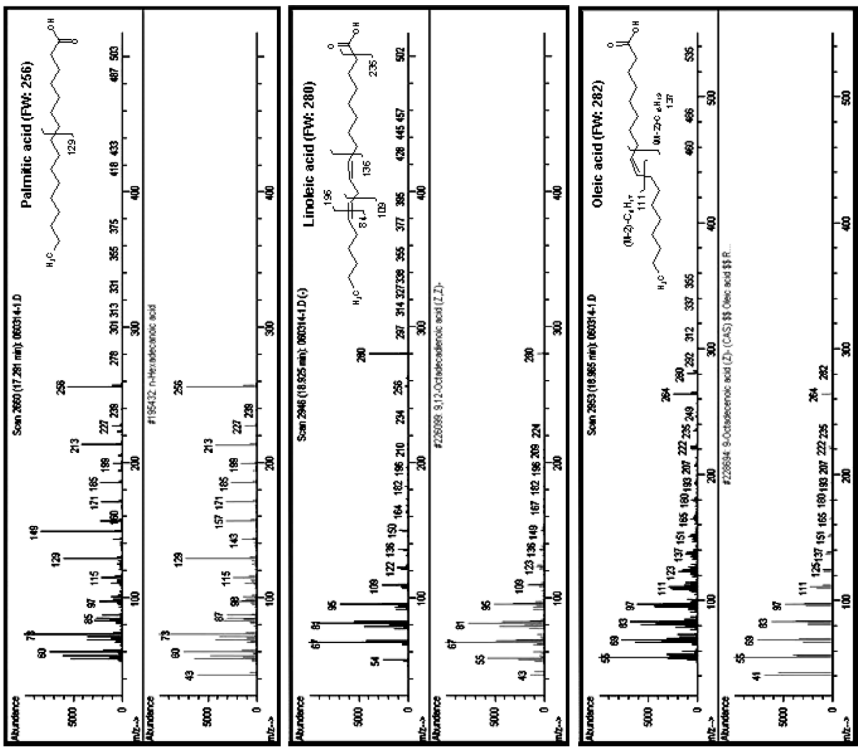

Fig. 4. Mass Spectra of Third Fraction Extracted from TLC Plate Separating MagS

The components in fraction 3 that exhibits bioactivity was analyzed by GC/MS. (A) Mass spectra of third fraction of MagS. (B, C, D) Mass spectra of major peak shown in panel A. Analyzed GC/MS data (top) arranged with standard mass profile of database (bottom). Commercial name, formula weight of identified fatty acid, and the structure of fatty acid with cleavage sites are described in inset.

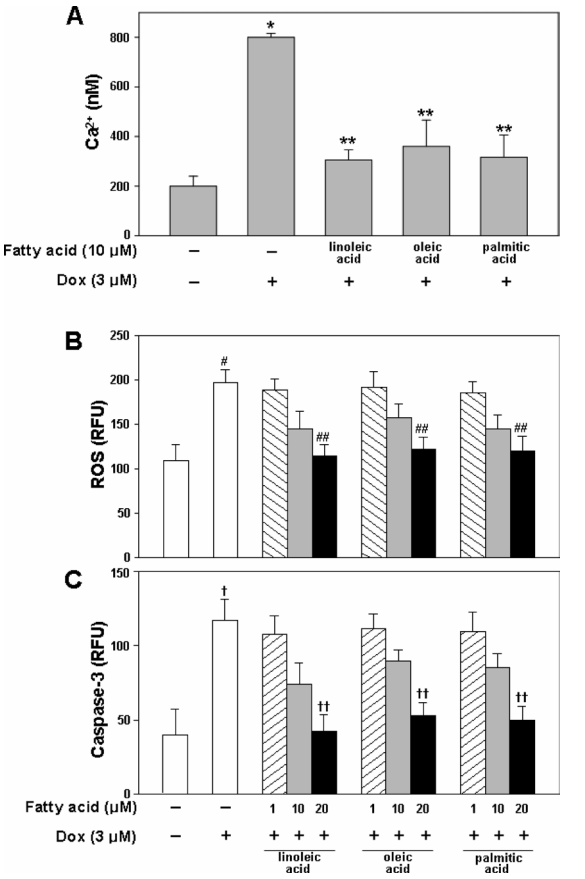

Fig. 5. Effect of Linoleic Acid, Oleic Acid and Palmitic Acid against Dox-Induced Toxicity in Rat Cardiomyocytes

A direct comparison of the Ca^{2+} level at 80 min (A), ROS generation (B), and caspase-3 activation (C) with 1 μM, 10 μM, and 20 μM of fatty acids. These data are presented mean±S.E.M. from three independent experiments in duplicate. $*p<0.01$ and $**p<0.05$; $\#p<0.01$ and $\#\#p<0.05$; $\dagger p<0.01$ and $\dagger\dagger p<0.01$ *versus* basal control (no drug treatment).

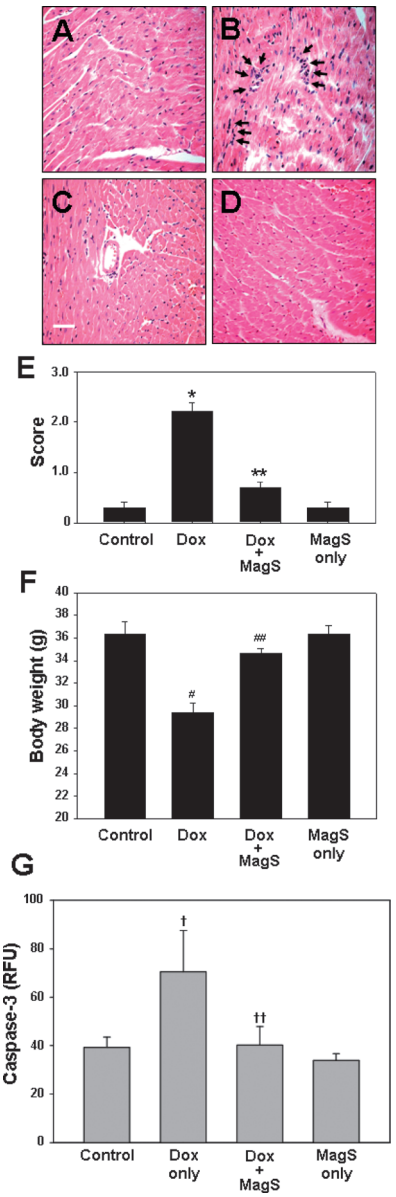

Fig. 6. MagS Reduces Cardiomyopathy and Decrease Body Weight in Mice Model

Hematoxylin-eosin staining of heart ventricle from BALB/c mice after treatment with or without Dox and MagS (A) Control mice, (B) Dox only, (C) Dox+MagS, (D) MagS only group. The infiltrating lymphocytes are observed in Dox only group while other groups are negative (black arrow). White scale bar=50 μm. (E) A relative score of the infiltrated lymphocyte compared from both group. (F) Effect of MagS against Dox-induced decrease of body weight. (G) Effect of MagS on caspase-3 activation by Dox administration. $*p<0.01$ and $**p<0.05$; $\#p<0.005$ and $\#\#p<0.01$; $\dagger p<0.05$ and $\dagger\dagger p<0.05$ *versus* basal control (no drug treatment).

whether these fatty acids exhibit detoxification ability to cardiomyocytes. Dox-induced Ca^{2+} increase was significantly reduced by pretreatment with 10 μM linoleic acid, oleic acid, and palmitic acid (Fig. 5A). Dox-induced ROS generation was also reduced significantly by these three fatty acids (Fig. 5B). Dox-induced caspase-3 activation was significantly inhibited by the fatty acids (Fig. 5C). These results suggest that fatty acids in MagS ameliorate Dox-induced cardiotoxicity.

MagS Reduces Dox-Induced Cardiotoxicity in Mice To further evaluate the *in vitro* results, we examined MagS *in vivo* using a mouse cardiomyopathy model induced by administration of Dox (Fig. 6). As expected administration of Dox resulted in cardiac tissue abnormality, showing lymphocyte infiltration and abnormal structure such as formation of vacuole and myofibrillar disarray (Fig. 6B). Upon treatment of Dox-treated mice with MagS, Dox-induced infiltration of lymphocytes and structural disarray of cardiac tissue were significantly reduced (Fig. 6C). MagS only showed no toxicity, showing similar results to control (Fig. 6D). MagS effects on Dox-induced cardiomyopathy including lymphocyte infiltration were summarized in Fig. 6E. In addition, treatment with Dox prevented gaining of body weights, losing body weight by 18.5±4.5% at 3 weeks later (Fig. 6F). In case of Dox+MagS group, a significant loss of body weight was not observed. However, the body weight of mice treated with MagS only group was increased as the control mice did. To assess the incidence apoptotic cell death in cardiac tissues, we determined the caspase-3 activity. The caspase-3 activity in the extracts of cardiac tissue of mice treated with MagS only was similar to that in the control. The enzyme activity in the extracts of hearts from Dox-treated mice was increased significantly compared to that in the control and the Dox-induced increase of the caspapse-3 activity was reduced significantly by treatment with MagS (Fig. 6G).

DISCUSSION

Dox has been in clinical use as an anti-cancer agent for a wide range of malignant tumors. However, its use has been limited by a dose-related and irreversible cardiotoxicity.[19] Dox-induced cardiotoxicity is known to be caused mainly by ROS generation. In addition, a disturbance of Ca^{2+} homeostasis by Dox is also implicated one of the cardiotoxic mechanisms.[4] Therefore, controlling ROS generation and maintaining Ca^{2+} homeostasis seem to be an approach for the prevention of cardiotoxicity induced by Dox.

Bioactive components from root and/or stem bark of *Magnolia* are known to contain bioactive components such as honokiol and magnolol.[16,20,21] In this study, we have investigated *Magnolia* seed extract to evaluate biological effects on Dox-induced cytotoxicity in *in vitro* and *in vivo*. Our results showed that MagS was able to inhibit Dox-induced increase in $[Ca^{2+}]_i$, ROS generation, and apoptosis in rat cardiomyocytes. Analysis of MagS revealed that the bioactive components in MagS were *n*-hexadecanoic acid (a class of palmitic acid), 9,12-octadecadienoic acid (a class of linoleic acid), and 9-octadecenoic acid, (a class of oleic acid). These fatty acids were able to inhibit Dox-induced increase in $[Ca^{2+}]_i$, ROS generation, and apoptosis in rat cardiomyocytes. Assessment of the ability of MagS *in vivo* revealed that Dox-induced cardiotoxicities such as infiltration of lymphocytes and disarray of cardiac muscle structure are significantly attenuated by treatment with MagS. In addition, several fatty acids have been shown to exhibit an anti-cancer activity by single or combination therapy with anthracyclins by increasing Dox cytotoxicity in human breast tumor cell line.[21,23] Although anticancer activity of MagS is unknown, MagS did not block Dox-induced Ca^{2+} increase in HL60 (leukemia), MDA-MB-231 (breast cancer), B16 (melanoma), PANC-1 (pancreatic cancer) (data not shown).

Studies have demonstrated that some fatty acids can attenuate cardiovascular diseases. Thus, Kang and Leaf have reported that eicosapentaenoic acid (EPA; 20 : 5n-3) and docosahexaenoic acid (DHA; 22 : 6n-3) exert protective effects against cardiovascular diseases such as arrhythmias generated by post-infarction.[24,25] The cardiopretective effect of these fatty acids is due to a reduction of ion channel activity by a direct interaction with the channel in plasma membranes. Consistent with the observations, EPA has also been shown to reduce voltage-gated L-type Ca^{2+} currents in adult and neonatal cardiomyocytes.[26] Vitelli *et al.* have suggested that DHA is capable of inhibiting Dox- or caffeine-induced release of Ca^{2+} from SR.[27] In the present study, our results showed that linoleic acid, oleic acid, and palmitic acid in MagS extract completely blocked Dox-induced Ca^{2+} increase at micromolar concentrations. We have previously reported that Dox induces Ca^{2+} release from SR *via* opening of ryanodine receptor.[4] In addition, studies have reported that Dox induces formation of vacuoles and disruption of myocardial architecture,[28—30] accumulation of lymphocytes,[31] including loss of body weight.[32] In agreement with these observations, our results revealed that Dox treatment resulted in disruption of myocardial architecture, infiltration of lymphocytes, and formation of vacuoles, including loss of body weight. All of these pathologic features were ameliorated by MagS.

In summary, fatty acids such as linoleic acid, oleic acid and palmitic acid in *Magnolia* seed exhibit protective effects on Dox-induced cardiotoxicities such as Ca^{2+} raise, ROS generation, and caspase-3 activation. Our results suggest that intake of fatty acids is recommendable for minimizing cardiac damage caused by Dox during cancer therapy.

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