ORIGINAL ARTICLE
Cardiovascular Disorders

JKMS

http://dx.doi.org/10.3346/jkms.2015.30.5.559 • J Korean Med Sci 2015; 30: 559-568

A Novel Angiotensin Type I Receptor Antagonist, Fimasartan, Prevents Doxorubicin-induced Cardiotoxicity in Rats

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Received: 16 June 2014
Accepted: 13 February 2015

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Funding: This study was supported by an investigator initiative research grant from Boryung Pharmaceutical, Inc., and by grants (Jeon ES, Lee YS) from the Korea Samsung Biomedical Research Institute (SBRI GL1-B2-271-1) (Jeon ES, Lim BK).

Angiotensin receptor blockers (ARBs) have organ-protective effects in heart failure and may be also effective in doxorubicin-induced cardiomyopathy (DOX-CMP); however, the efficacy of ARBs on the prevention of DOX-CMP have not been investigated. We performed a preclinical experiment to evaluate the preventive effect of a novel ARB, fimasartan, in DOX-CMP. All animals underwent echocardiography and were randomly assigned into three groups: treated daily with vehicle (DOX-only group, n = 22), 5 mg/kg of fimasartan (Low-fima group, n = 22), and 10 mg/kg of fimasartan (High-fima group, n = 19). DOX was injected once a week for six weeks. Echocardiography and hemodynamic assessment was performed at the 8th week using a miniaturized conductance catheter. Survival rate of the High-fima group was greater (100%) than that of the Low-fima (75%) and DOX-only groups (50%). Echocardiography showed preserved left ventricular (LV) ejection fraction in the High-fima group, but not in the DOX-only group ($P = 0.002$). LV dimensions increased in the DOX-only group; however, remodeling was attenuated in the Low-fima and High-fima groups. Hemodynamic assessment showed higher dP/dt in the High-fima group compared with the DOX-only group. A novel ARB, fimasartan, may prevent DOX-CMP and improve survival rate in a dose-dependent manner in a rat model of DOX-CMP and could be a treatment option for the prevention of DOX-CMP.

Keywords: Angiotensin Receptor Blocker; Doxorubicin-induced Cardiomyopathy

INTRODUCTION

Doxorubicin (DOX) is the most commonly used chemotherapeutic agent and is frequently included in chemotherapy regimens for the treatment of lung, breast, stomach, ovarian, thyroid cancer, soft tissue sarcoma multiple myeloma and some leukemias and Hodgkin's lymphoma.

The acute adverse effects of DOX, such as nausea, vomiting, alopecia, and neutropenia, are usually nonfatal or reversible. However, cardiotoxicity is the most serious sequelae of DOX-based chemotherapy (1).

Cardiotoxicity is associated with cumulative doses of DOX. An empirical dose limit of 500 mg per m^2 is recommended as the cut-off value to minimize DOX-induced cardiotoxicity (2). Several clinical risk factors have been implicated in DOX-induced cardiomyopathy (3, 4). However, even in patients without any risk factors, development of DOX-induced cardiotoxicity has been reported (1). Therefore, monitoring cumulative doses of DOX may fail to prevent cardiac toxicity, which can occur unexpectedly. The mortality and morbidity of DOX-induced dilated cardiomyopathy are high and often irreversible; therefore, preventive management and cautious monitoring are needed during the chemotherapy. Even with the cardiac risks associated with DOX, it is still included in many chemotherapy regimens because of its efficacy in various cancers.

Preventive management of DOX-induced cardiomyopathy mainly includes close monitoring during treatment and early termination when cardiotoxicity is suspected (5); however, deterioration is often observed even after termination of chemotherapy. Experimental and human data suggest that anti-oxidative treatment or angiotensin-converting enzyme/angiotensin-receptor antagonists may have protective effects. In this study, we investigated whether pretreatment with two different doses of a new angiotensin-receptor antagonist, fimasartan, could prevent DOX-induced cardiotoxicity and improve survival rates.

MATERIALS AND METHODS

Study design

The protocols used in this study conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication 85-23, revised 1996). A total of 71 eight-week-old Sprague-Dawley rats weighing 250-300 g was used (Fig. 1). Echocardiography was performed in all ani-

pISSN 1011-8934
eISSN 1598-6357

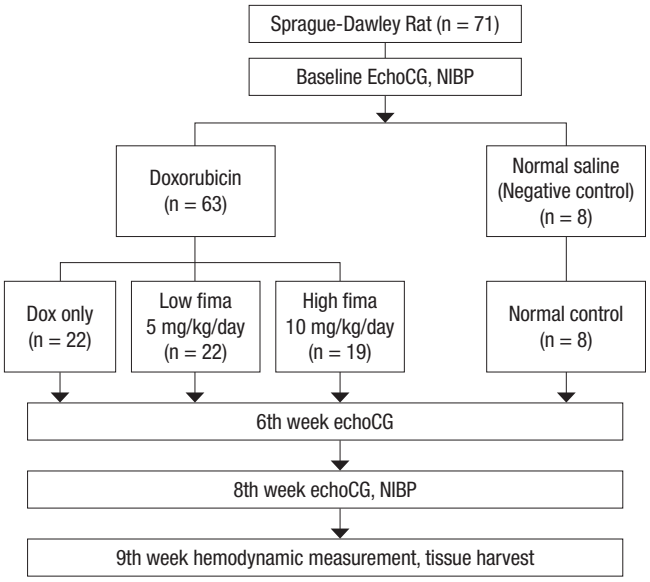

Fig. 1. Study design. After baseline echocardiography (EchoCG), animals were randomly assigned into two groups: negative control (NC) and DOX (DOX-only; 3 mg/kg of DOX intravenously once a week for six weeks) groups. The DOX-only group was divided into three groups as follows: non-treated (DOX-only), low-dose fimasartan (Low-fima), and high-dose fimasartan (High-fima). Echocardiography and NIBP was performed in all the study animals at weeks six and eight from baseline, and hemodynamic evaluation was performed at week nine from baseline.

mals before enrollment to acquire baseline echocardiographic data. Animals with normal LV function were randomly assigned into two groups: a negative control group (n = 8) and a DOX group (n = 63). The DOX group was divided into three subgroups: DOX-only without fimasartan (DOX, n = 22), low-dose fimasartan (Low-fima, 5 mg/kg/day, n = 22), and high-dose fimasartan (High-fima, 10 mg/kg/day, n = 19). DOX (3 mg/kg/day, doxorubicin [DOX 44583], Sigma Aldrich, St. Louise, MO, USA) was injected intravenously via the tail vein to all animals in the DOX-only group once a week for six weeks from the time of randomization. Animals injected with saline were used as negative controls (n = 8).

Fimasartan (Kanarb, Boryung Inc., Seoul, South Korea) was mixed with 0.5% carboxy methyl cellulose (CMC) to make a suspension. All fimasartan-treated groups were administered daily by gastric gavage for nine weeks with a dose of 5 mg or 10 mg/kg/day as described above. DOX-only and normal control (NC) groups were treated with vehicle (CMC) only.

Echocardiography and non-invasive blood pressure (NIBP) measurement were performed at baseline and six and eight weeks after treatment. At week nine from baseline, hemodynamic measurement and tissue harvest were performed consecutively.

Systolic and diastolic blood pressures of conscious rats were measured by the tail-cuff method using commercialized equipment (IITC Life Science Inc., CA, USA) at baseline and eight weeks. Non-invasive blood pressure monitoring was performed one day before echocardiographic examination to minimize the stress imposed on the animals. Body weight was measured every week from baseline through week nine. The general condition and mortality of rats were monitored daily and a survival curve was derived.

Echocardiography

Echocardiography was performed at baseline, six weeks, and eight weeks. The rats were anesthetized by the 1.5% isoflurane inhalation method with nosecone. Images were acquired with a 12 MHz linear transducer connected to a Vivid-7 echocardiography machine (GE Medical, Milwaukee, WI, USA). M-mode and two-dimensional echocardiography images were acquired at the papillary muscle level with a frame rate of 80-120/sec. LV end-diastolic septal and posterior wall thickness (SWT and PWT), LV end-diastolic dimension (LVEDD), and LV end-systolic dimension (LVESD) were measured. The LV ejection fraction (LVEF) was calculated according to the following formula: LVEF = (LVEDD^3-LVESD^3)/LVEDD^3. A single echocardiographer who was blinded to the treatment information performed all echocardiograms for data acquisition.

Hemodynamic measurements

One week after echocardiography (week nine after treatment), rats were intubated with a 16-gauge catheter after induction of anesthesia with 5% isoflurane. Anesthesia was maintained with 1.5% isoflurane and the animals were placed in the recumbent position on a heat pad with a rectal probe connected to a thermoregulator. The animals were then intubated with a blunt 16-gauge needle using the tracheotomy method and ventilated with a custom-designed constant-pressure ventilator at 75 breaths/min using room air. An anterior thoracotomy was performed and a small apical stab was made to expose the LV apex. Electrocautery was used to minimize bleeding during the surgical procedure.

After the apex of the LV was stabbed with a 27-gauge needle, a micro tip pressure-volume (P-V) catheter (SPR-838, Millar Instruments; Houston, TX, USA) was inserted retrograde into the LV cavity along the cardiac longitudinal axis until stable P-V loops were obtained (6). Polyethylene catheters (PE-50) were inserted into the right femoral artery for measurement of mean arterial pressure. The right internal jugular vein was used as a central venous line for fluid administration. The abdominal wall was opened, and the inferior vena cava (IVC) and portal vein were exposed. A snare suture was placed to modulate rapid IVC obstruction. All loops were acquired after 20 min of stabilization with the ventilator turned off for 5-10 sec. The sampling rate was 1,000/s using the ARIA P-V conductance system (Millar Instruments) coupled to a PowerLab 16/30 A/D converter (AD Instruments; Mountain View, CA, USA) and a personal computer. After the data were recorded under steady state and preload reduction by IVC ligation, parallel conductance (Vp) was

 http://dx.doi.org/10.3346/jkms.2015.30.5.559

obtained by injecting 500 μL of 15% hypertonic saline into the central venous line.

Volume calibration was performed using the electrical cuvette method. Electrical cuvettes (Millar Instruments) were filled with 500 μL of fresh blood and blood viscosity was determined. After acquisition of V_p at the end of hemodynamic evaluation, volume correction with V_p was performed and loops were analyzed using a commercially available cardiac P-V analysis program, PVAN Ultra V1.0.2 (Millar Instruments). Heart rate, LV end-diastolic pressure (EDP), maximal slope of systolic pressure increment (+dP/dt) and diastolic pressure decrement (-dP/dt), LVEF, end-systolic and end-diastolic volume (ESV and EDV), and stroke volume were calculated.

LV P-V relations were measured via transient occlusion of the IVC with a silk snare suture. Successive cardiac cycles (10-20) were obtained over 5 sec, from which the end-systolic pressure volume relation (ESPVR) slope, SW-EDV relation (preload recruitable stroke work [PRSW]), slope of the maximum first derivative of ventricular pressure with respect to time (dP/dt_{max})-EDV relation, and end-diastolic pressure volume relation (EDPVR) slope were derived.

Western blot analysis

For Western blot analysis, heart was lysed in RIPA buffer (50 mM Tris [pH 8.0], 0.1% SDS, 1% NP40, 150 mM NaCl, 0.5% sodium-deoxycholate). Aliquots of total heart extract (10 μg) were loaded onto 12% SDS-PAGE gels, electrophoresed for 4 hr at 100 V, and then transferred to Hybond-ECL nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ, USA). The membranes were blocked in 5% non-fat dry milk solution in phosphate-buffered saline (PBS) containing 0.1% Tween 20 and then probed with commercial antibodies to phospho-ERK (extracellular signal-regulated kinase), total ERK, phospho-AKT, and total AKT (1:1,000 rabbit polyclonal antibody, Cell Signaling, CA, USA). After incubation in ECL solution, membranes were exposed to x-ray film.

Statistical analysis

Data were reported as means and standard deviations. One way analysis of variance (ANOVA) with post-hoc analysis or Kruskal-Wallis test was used to compare mean values of the groups where appropriate. Kaplan-Meier survival curves were constructed and log-rank tests were performed. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed with IBM SPSS Statistics (Version 19.0, IBM SPSS Inc., Chicago, IL, USA).

Ethics statement

All procedures were reviewed and approved by the institutional animal care and use committee of Samsung Biomedical Research Institute (SBRI) [IRB No. S-B1-005]. SBRI is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and abides by the Institute of Laboratory Animal Resources guide.

RESULTS

There were no differences among the groups in baseline characteristics, including blood pressure or echocardiographic findings (Table 1). Mean blood pressure in the High-fima group was significantly decreased at eight weeks (80.8 ± 7.3 mmHg) compared to the NC group (108.0 ± 9.2 mmHg, $P = 0.01$) and the DOX-only group (97.0 ± 6.9 mmHg, $P = 0.001$, Fig. 2A). Body weight increased until week three from baseline, and then decreased from weeks four to nine in all DOX-only animals (Fig. 2B). In contrast, rats in the NC group showed gradually increasing body weight until the end of the study. At eight weeks, body weights in both fimasartan-treated groups (Low-fima 320.3 ± 40.7 g, High-fima 317.2 ± 34.0 g) were higher than those in the DOX-only group (279.3 ± 50.4 g, Fig. 2B).

Echocardiographic data showed progressive LV systolic dysfunction and dilation of the LV cavity in the DOX-only group (Fig. 2C and D, Table 2). Specifically, LVESD at six weeks was maintained until end of the study in both the High-fima (4.94 ± 1.08

Table 1. Baseline characteristics of study animals

Parameters	Doxorubicin group			Normal control (n = 8)	*P* value
	Dox-only (n = 22)	Low-fima (n = 22)	High-fima (n = 19)		
Body weight (g)	310.7 ± 29.3	310.3 ± 30.3	308.6 ± 29.7	300.1 ± 25.4	0.837
Mean BP (mmHg)*	103.8 ± 7.7	100.7 ± 8.9	98.9 ± 7.0	97.6 ± 7.4	0.177
Systolic BP (mmHg)*	126.5 ± 7.2	123.0 ± 9.7	122.0 ± 7.9	124.3 ± 6.8	0.217
Diastolic BP (mmHg)*	93.1 ± 7.8	90.9 ± 10.6	89.8 ± 8.3	87.3 ± 8.2	0.534
HR (beats/min)	364.2 ± 52.0	350.5 ± 83.0	357.8 ± 56.6	348.2 ± 45.7	0.788
LV EF (%)	75.8 ± 4.0	76.0 ± 3.0	76.3 ± 2.6	77.4 ± 4.2	0.883
LV EDD (mm)	7.08 ± 0.63	7.29 ± 0.52	6.90 ± 0.46	7.08 ± 0.66	0.075
LV ESD (mm)	4.26 ± 0.51	4.38 ± 0.40	3.93 ± 0.92	4.16 ± 0.59	0.075
LV SWT (mm)	1.14 ± 0.12	1.10 ± 0.20	1.14 ± 0.16	1.15 ± 0.14	0.629
LV PWT (mm)	1.28 ± 0.17	1.35 ± 0.15	1.29 ± 0.20	1.48 ± 0.17	0.329

Values area means ± SD. *Measured by noninvasive blood pressure. BP, blood pressure; HR, heart rate; LV, left ventricular; EF, ejection fraction; EDD, end diastolic dimension; ESD, end systolic dimension; SWT, diastolic posterior wall thickness; PWT, diastolic posterior wall thickness.

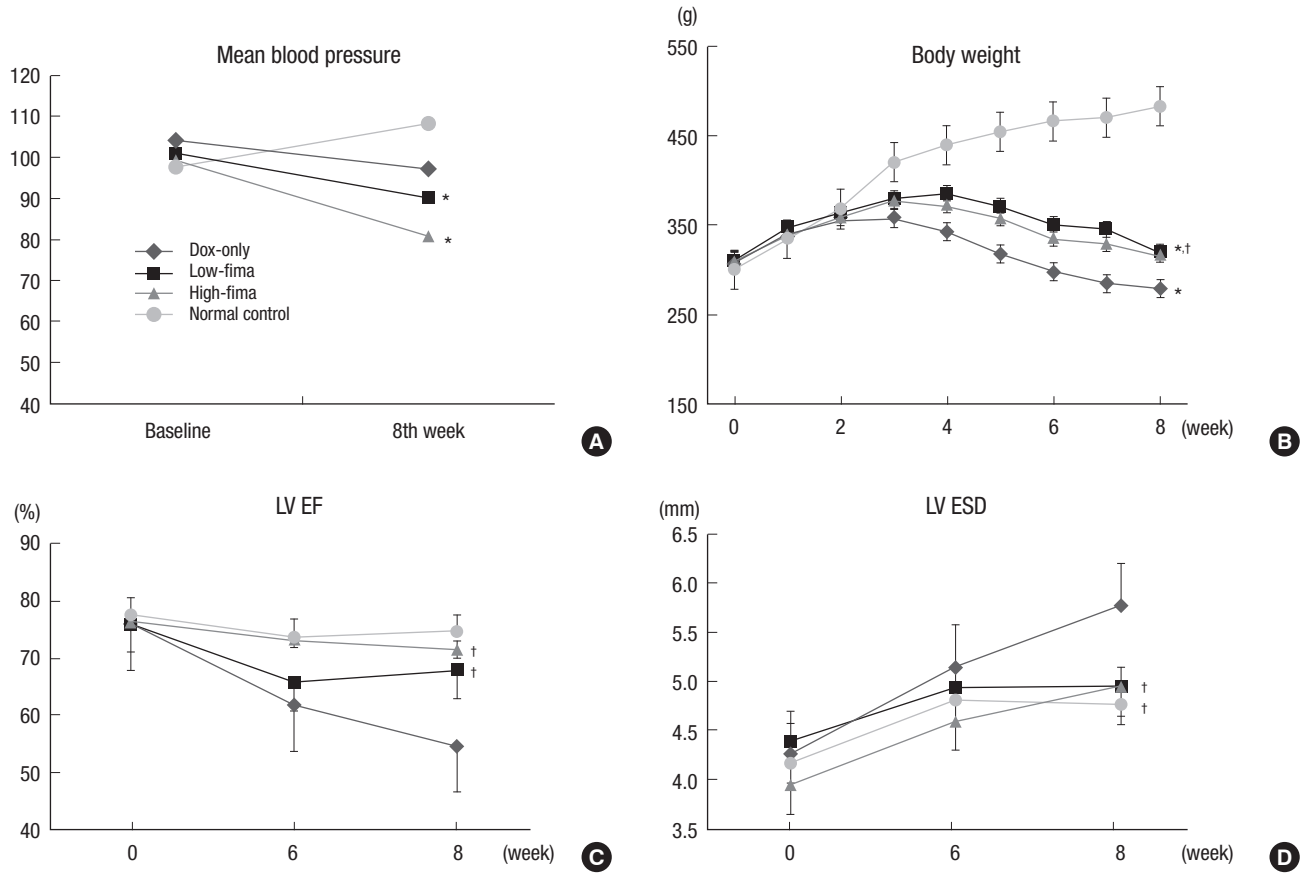

Fig. 2. Changes in blood pressure, body weight, and echocardiographic findings. (**A**) Mean blood pressure was significantly decreased in the fimasartan-treated group (High-fima and Low-fima) compared with the normal control group at week eight from baseline. (**B**) Body weight increased in all animals until week three from baseline, and then decreased in all animals administered with DOX. At eight weeks, the DOX-only group showed the lowest body weight. (**C**) LVEF also decreased in the DOX-only group at eight weeks. In contrast, body weight and LVEF were preserved in High-fima and Low-fima groups. (**D**) LV end-systolic dimension (ESD) progressively increased in the DOX-only group; however, LV dilatation was attenuated in Low-fima and High-fima groups (*$P < 0.05$ compared to NC group, †$P < 0.05$ compared to DOX-only group).

Table 2. Echocardiographic data after 6 and 8 weeks of treatment

Parameters	Doxorubicin group			Normal control (n = 8)	*P* value
	Dox-only (n = 22)	Low-fima (n = 22)	High-fima (n = 19)		
At 6 weeks					
HR (beats/min)	304.6 ± 52.0	334.5 ± 49.2	322.9 ± 84.3	352.7 ± 46.6	0.316
LV EF (%)	61.7 ± 12.7	65.7 ± 14.9	73.2 ± 4.8*	73.6 ± 4.1	0.013*
LV EDD (mm)	7.48 ± 0.57	7.48 ± 0.63	7.46 ± 0.59	7.77 ± 0.92	0.993
LV ESD (mm)	5.14 ± 0.56	4.93 ± 0.67	4.59 ± 0.65*	4.81 ± 0.71	0.032*
LV SWT (mm)	1.21 ± 0.20	1.11 ± 0.12	1.20 ± 0.13	1.30 ± 0.05	0.080
LV PWT (mm)	1.29 ± 0.23	1.35 ± 0.21	1.21 ± 0.17	1.38 ± 0.20	0.130
At 8 weeks					
HR (beats/min)	280.1 ± 40.2	329.8 ± 42.2*	346.0 ± 36.0*	354.4 ± 53.6	< 0.001*
LV EF (%)	54.6 ± 8.4	67.9 ± 5.3*	71.4 ± 6.3*	74.6 ± 3.7	< 0.001*
LV EDD (mm)	7.76 ± 0.41	7.42 ± 0.47*	7.30 ± 0.71	7.76 ± 0.53	0.077
LV ESD (mm)	5.60 ± 0.60	4.95 ± 0.52*	4.94 ± 1.08*	4.76 ± 0.49	0.009*
LV SWT (mm)	1.12 ± 0.13	1.18 ± 0.19	1.17 ± 0.14	1.36 ± 0.16	0.577
LV PWT (mm)	1.24 ± 0.14	1.24 ± 0.11	1.26 ± 0.13	1.50 ± 0.17	0.921

Values area means ± SD. *P value < 0.05 by ANOVA among doxorubicin injected group. HR, Heart rate; LV, left ventricular; EF, ejection fraction; EDD, end diastolic dimension; ESD, end systolic dimension; SWT, diastolic posterior wall thickness; PWT, diastolic posterior wall thickness.

mm) and Low-fima (4.95 ± 0.52 mm) groups; however, LVESD in the DOX-only group was significantly increased compared with the fimasartan-treated groups (56 ± 0.6 mm, $P = 0.009$ by ANOVA). Furthermore, LVEF was preserved in the High-fima group (71.4% ± 6.3%) and slightly decreased in the Low-fima group (67.9% ± 5.3%) compared with the NC group (74.6% ± 3.7%).

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In contrast, LVEF was significantly decreased in the DOX-only group (54.6% ± 8.4%, $P < 0.001$ by ANOVA).

Only 55% of the DOX-only group survived to nine weeks before hemodynamic assessment. The Low-fima and High-fima groups had survival rates of 77% and 95%, respectively. All of the animals in the normal control group were alive at the end of the study (Fig. 3, $P = 0.002$ by log-rank test).

Diarrhea and abdominal distension associated with a large amount of ascites were common in the DOX-only group (n = 15 [68%] and n = 11 [50%], Table 3); however, these conditions did not occur in the High-fima group. A small number of animals (n = 6 [27%]) in the Low-fima group had diarrhea. Although none of the fimasartan-treated rats showed abdominal distension, a small amount of ascites was found in about a quarter of the rats treated with fimasartan when the abdominal walls were opened and the visceral space examined. Body weight at eight weeks was smallest in the DOX-only group. Severe anorexia, she-

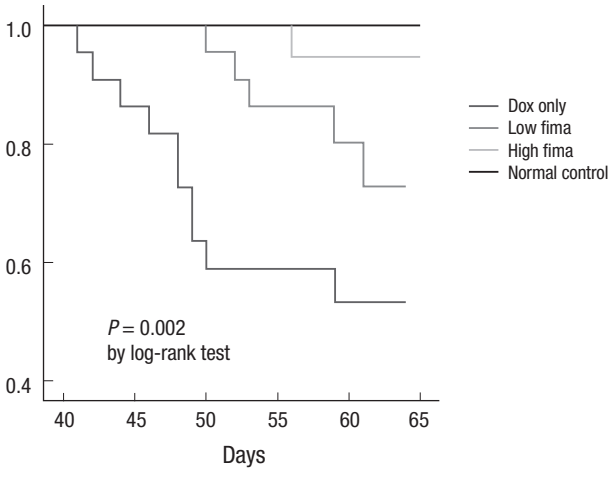

Fig. 3. Survival curves and general features of study animals. Eight-week survival rate of the High-fima group is greater (100%) than that of Low-fima (75%) and DOX-only groups (50%, $P < 0.05$).

Table 3. General conditions of study animals

Conditions	Doxorubicin group			Normal control (n = 8)	*P* value
	Dox-only	Low-fima	High-fima		
Body weight (8th week)*	279.3 ± 50.4	320.3 ± 40.7	317.2 ± 34.0	482.8 ± 52.4	< 0.001
Eye discharge/conjunctival hemorrhage (No.)	7	0	0	0	< 0.001
Diarrhea (No.)	15	6	0	0	
Abdominal distension (No.)	11	0	0	0	< 0.001
Ascites (pathology) (No.)*	10	4	4	0	< 0.001

*Evaluation for body weight and ascites was performed at the end of the study, therefore number of evaluated animals is as follows: Dox-only, n = 12; Low-fima, n = 19; High-fima, n = 19; normal control, n = 8.

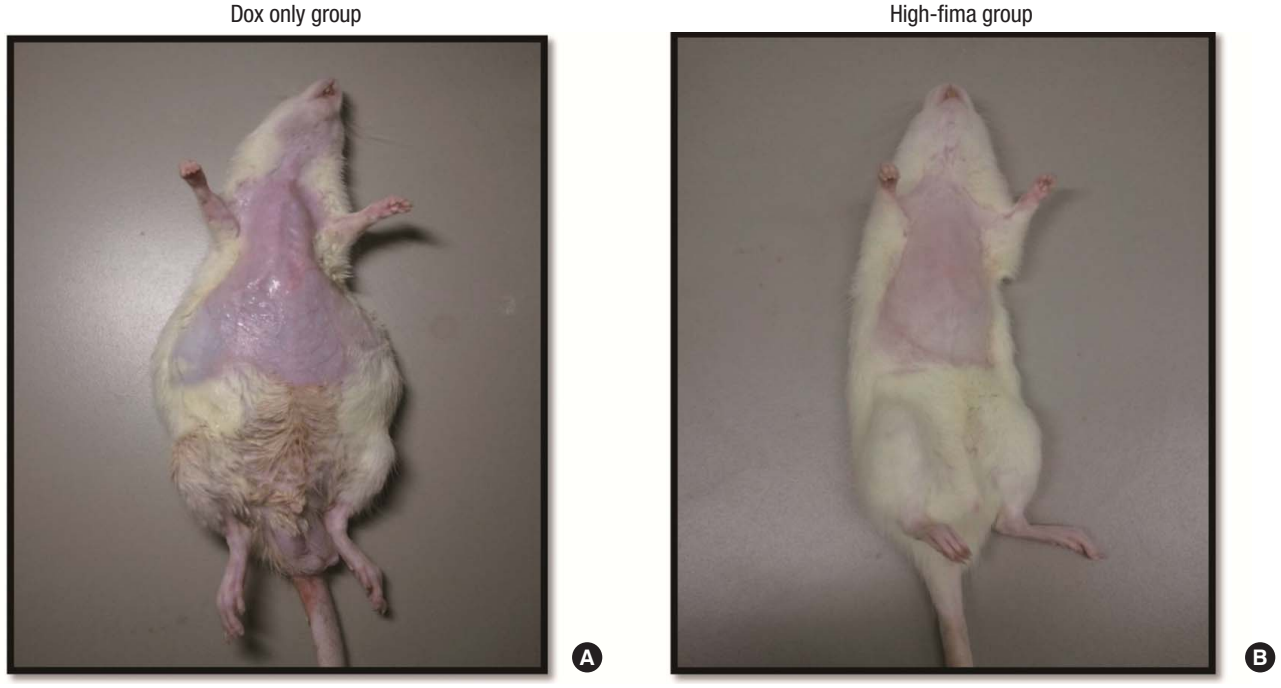

Fig. 4. General feature of treated animals. (**A**) Rats in Dox-only group show severe shedding and ascites. (**B**) In contrast, rats in the High-fima group show no shedding or ascites.

dding, and eye discharge with conjunctival hemorrhage were also common in the DOX-only group . In contrast, rats in the High-fima group showed less anorexia and no shedding, eye discharge, or ascites (Table 3, Fig. 4).

Table 4. Hemodynamic result at 9 weeks

Parameters	Doxorubicin group			Normal control (n = 8)	*P* value
	Dox-only (n = 10)	Low-fima (n = 15)	High-fima (n = 13)		
Heart rate (beat/min)	237.5 ± 37.5	267.5 ± 30.9	266.9 ± 29.7	286.3 ± 63.5	0.057
LV EDP (mmHg)	9.2 ± 3.5	9.0 ± 2.5	8.9 ± 3.3	9.0 ± 2.5	0.983
+dP/dt	3,425.3 ± 947.1	4,241.1 ± 888.4	5,931.6 ± 1,354.2†‡	6,146.6 ± 1,439.0	< 0.001*
-dP/dt	3,121.9 ± 1,127.5	3,502.1 ± 1,088.1	5,410.3 ± 1,338.2†‡	5,406.1 ± 1,074.6	< 0.001*
Ees	0.27 ± 0.18	0.25 ± 0.23	0.35 ± 0.23	0.31 ± 0.23	0.534
Slope –EDPVR	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.04	0.02 ± 0.01	0.609
Emax	0.89 ± 0.41	1.01 ± 1.67	0.99 ± 5,494	1.96 ± 2.57	0.210
PRSW	44.0 ± 14.5	52.3 ± 24.8	53.8 ± 50.6	57.1 ± 25.7	0.609
dP/dt-EDV	11.2 ± 9.5	12.3 ± 12.9	26.9 ± 21.5	28.9 ± 17.3	0.042*

*P value < 0.05 by ANOVA among doxorubicin injected group; †P value < 0.05 by post poc analysis compared to Dox-only group; ‡P value < 0.05 by post poc analysis compared to low-fima group. LV, left ventricular; ESV, end systolic volume; EDV, end-diastolic volume; τ, tau; Ees, end-systolic pressure-volume relation; Emax, maximum elastance; EDPVR, end-diastolic pressure volume relation; PRSW, preload recruitable stroke work.

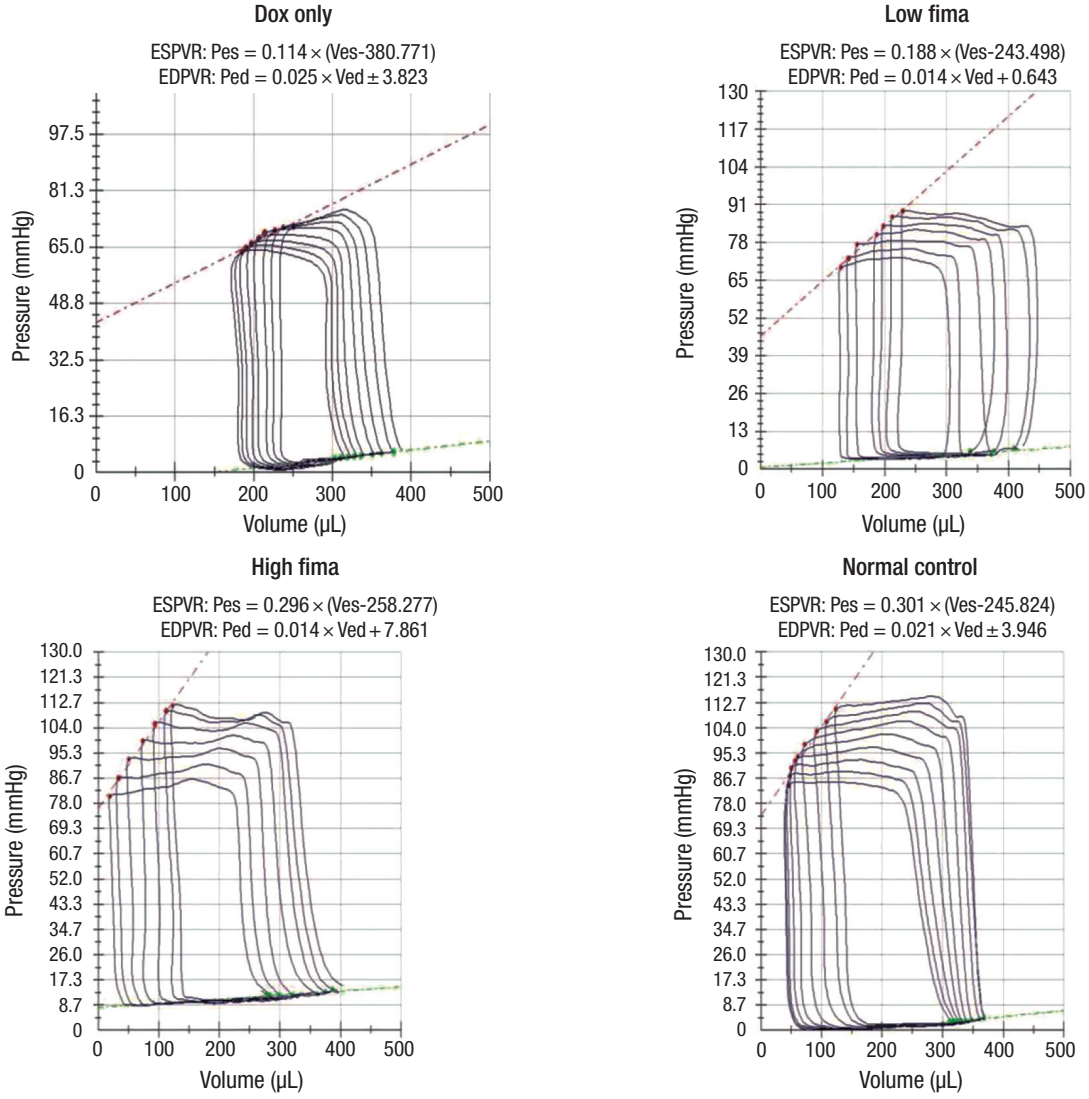

Fig. 5. Left ventricular pressure-volume loop by microminiaturized press-volume catheterization. End-systolic pressure volume relation (ESPVR) slopes was significantly decreased in DOX-only and Low-fima groups, whereas slope values was similar to normal control in the High-fima group.

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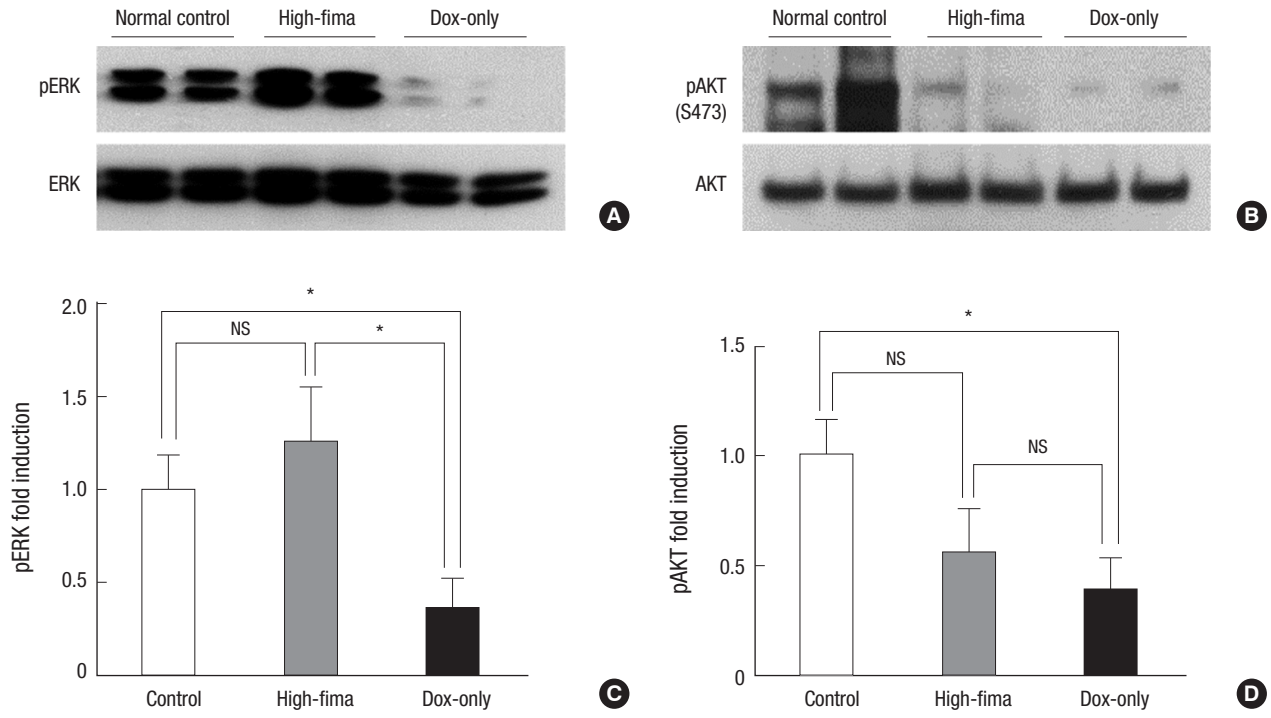

Fig. 6. ERK and AKT activity in week nine heart lysates by Western blot analysis. Decreased ERK phosphorylation is reversed by high-dose fimasartan treatment (**A**, **C**). However, AKT phosphorylation is not affected, suggesting that fimasartan may activate cell survival signaling under DOX-induced cardiotoxicity (**B**, **D**). *$P < 0.05$. NS, not significant.

Hemodynamic data acquired using a micro-conductance catheter are summarized in Table 4 and Fig. 5. The absolute values of positive and negative dP/dt were highest in the High-fima group (5,931 ± 1,354 mmHg/sec and -5,410 ± 1,338 mmHg/sec) compared with the Low-fima (4,241 ± 888 mmHg/sec and -3,502 ± 1,088 mmHg/sec) and DOX-only groups (3,425 ± 947 mmHg/sec and -3,121 ± 1,127 mmHg/sec, $P < 0.001$ by ANOVA, respectively). However, other parameters such as Ea, Ees, Emax, and PRSW did not show statistically significant differences between the groups. dP/dt-EDV was significantly higher in the High-fima group, with similar values to normal controls. The end-systolic pressure volume relation (ESPVR) slopes were significantly decreased in the DOX-only and Low-fima groups, whereas slope values were similar to normal control in the High-fima group (Fig. 5).

Western blot analysis showed that pERK protein levels were decreased in the DOX-only group and increased in the High-fima group compared with the normal control group (Fig. 6A). In addition, pAKT protein levels were decreased both in the DOX-only group and in the High-fima group (Fig. 6B). There was a remarkable increase in pERK in the High-fima group compared with the DOX-only group (Fig. 6C). The expression of pAKT was significantly decreased compared to the normal control group and was decreased in the High-fima group without statistical significance (Fig. 6D). These findings suggest that fimasartan may activate cell survival signaling under Doxorubicin-induced cardiotoxicity.

DISCUSSION

In this study, a new ARB, fimasartan, prevented progressive DOX-induced cardiac dysfunction in a rat model of DOX-induced cardiomyopathy when administrated during DOX treatment. In addition this ARB also attenuated the systemic toxicity of DOX and improved the survival of rats in a dose-dependent manner.

Treatment for DOX-induced cardiomyopathy has been studied with respect to prevention or treatment after development of cardiomyopathy. The delayed occurrence of cardiomyopathy, even after as long as ten years (7), suggests that initial cardiac injury by DOX lasts for years, resulting in progressive irreversible damage to the myocardium. Therefore, pharmacological therapy for prevention should be initiated before the administration of DOX and continued for a long period of time, even after discontinuation of chemotherapy. The mechanism of DOX-induced cardiomyopathy is the production of free radicals and a decrease in endogenous antioxidant enzymes, which results in tissue-specific mitochondrial DNA damage induced by oxidative stress (7, 8). Therefore, antioxidant drugs are expected to be a possible preventive method for minimizing the toxic effects of DOX. Several pharmaceutical agents acting on oxidative stress have been evaluated in preclinical studies, and a few clinical trials have shown success (9, 10). However, the effect was limited and a risk of other adverse events such as secondary malignancy was reported.

The therapeutic effects of the long-term use of beta blockers, angiotensin converting enzyme inhibitor, or ARB have been established in patients with systolic heart failure in many clinical trials (11-13), and these drugs are expected to have a similar effect on heart failure from other causes. Carvedilol (14) and enalapril (15) were tested for the treatment of DOX-induced cardiomyopathy in preclinical and clinical studies with a small number of patients. In our data, high dose of fimasartan showed the superior effect on survival and hemodynamics than low dose of fimasartan. In previous clinical studies with ARB in heart failure or renal failure, dose dependent manner was observed (16, 17). It can be class-effect of ARB but further clinical study is needed in fimasartan.

The renin-angiotensin system plays a crucial role in the cardiovascular system, and angiotensin II is closely related to cardiac injury and the progression of ventricular remodeling in heart failure. Angiotensin II type 1 (AT1) receptor is especially associated with cellular proliferation, vasoconstriction, and sympathetic activation. The blockage of AT1 receptor with ARB has been reported to modulate neurohormonal activation and have antioxidative effects. Moreover, DOX-induced cardiomyopathy is not observed in AT1 receptor knock-out mice (18). Thus, AT1 receptor may play a role in the progression of DOX-induced cardiomyopathy. Several preclinical studies with ARB (19-21) showed the protective or therapeutic effects of ARB in a DOX-induced cardiomyopathy model via anti-inflammatory processes or a decrease in oxidative stress.

Fimasartan (BR-A-657; BR-A-657-K; Kanarb®) is a new angiotensin II receptor antagonist with high selectivity for the AT1 receptor subtype. Fimasartan shows superior inhibitory activity in the contraction of isolated rabbit thoracic aorta compared with other ARBs such as losartan and candesartan (22) and showed protective effect in acute myocardial infarction model in a recent study (23). In clinical trials with hypertensive patients, fimasartan shows non-inferiority to losartan for blood pressure control (22). Fimasartan has not previously been studied for the treatment of heart failure; however, it is expected to have an ARB class effect on heart failure, and its efficacy may be greater than expected because of its high affinity for AT1 receptor compared with other ARBs in preclinical studies.

In our study, the systemic toxicity of DOX was dramatically decreased by fimasartan treatment. The decreased toxicity may have been mediated by the ERK pathway, which is associated with cell survival, via an induction of ERK phosphorylation by high-dose fimasartan treatment. Another signaling pathway associated with cell survival, the AKT phosphorylation pathway, was not affected by fimasartan treatment. Damaged myocyte increase angiotensin II secretion and it is activating JAK-STAT pathway. Activated STAT signal pathway was negatively regulated MAPK signal. It could increase myocyte death. Thus DOX induce myocyte damage was significantly protected by fimasartan induce ERK signal cascade activation (24-28).

Potential cardiotoxicity by chemotherapeutic agents may last for years, resulting in insidious onset of overt cardiomyopathy after years of latency (29). Therefore, pharmacological therapy for prevention should be initiated at the time of DOX administration and be continued for a long period, even after the discontinuation of the chemotherapy. For this purpose, preventive agents should have long-term safety, tolerability, and low cost. Moreover, preventive medication should not alter the anti-tumor effects of DOX.

In this regard, ARBs are valuable pharmacological agents that can be used to prevent DOX-induced cardiomyopathy. The safety of ARBs has been well-studied in many clinical trials. Apart from other antihypertensive medications, incremental doses of ARBs decrease blood pressure, but rarely induce an abnormal reduction in blood pressure (11). In many clinical trials evaluating ARBs in hypertensive or heart failure patients, the adverse effects of ARBs were found to be minimal (11, 14, 22). The daily cost of ARBs can be managed by most patients when compared with the cost of chemotherapy and the fatal events that may occur when patients develop heart failure.

Our study showed that fimasartan had a strong preventive effect on DOX-induced cardiomyopathy. Moreover, fimasartan improved survival rates and systemic toxicity. These findings suggest that fimasartan may be a possible candidate to reduce adverse reactions during DOX-based chemotherapy such as cardiac and other systemic toxicity.

Our study did not evaluate the anti-tumor effects of fimasartan. In a previous study, AT1 receptor was shown to be associated with cellular proliferation; thus, ARBs have been reported to have anti-proliferative activity and anti-tumor effects (30). Therefore, the possibility of fimasartan treatment altering the anti-tumor effects of DOX is very low. The dosage of fimasartan used in this study was high in order to decrease the blood pressure of the study animals; therefore, this could be a possible limiting factor in clinical use. However, most ARBs have shown tolerable blood pressure reduction in clinical trials of patients with heart failure (11, 14).

In conclusion, a new ARB, fimasartan, when administrated during DOX treatment, prevented progressive DOX-induced cardiac dysfunction in a rat model of DOX-induced cardiomyopathy. In addition, fimasartan also attenuated the systemic toxicity of DOX and improved rat survival in a dose-dependent manner. These data demonstrate the possibility of fimasartan as a treatment option to prevent DOX-induced cardiac dysfunction and heart failure. For that reason, randomized clinical trials with fimasartan are warranted in the near future.

DISCLOSURE

The authors have no potential conflicts of interest to declare in

http://dx.doi.org/10.3346/jkms.2015.30.5.559

relation to this article.

AUTHOR CONTRIBUTIONS

Conceived and designed the study: Chang SA, Lim BK, Jeon ES. Data collection: Chang SA, Lee YJ, Hong MK, Lim BK. Analyzed the data: Chang SA, Lim BK, Choi JO. Statistical analysis: Chang SAS, Lim BK. Manuscript preparation: Chang SA, Lim BK, Choi JO, Jeon ES. Manuscript approval: all authors.

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