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ORIGINAL ARTICLE

Beneficial effects of angiotensin II receptor blocker, olmesartan, in limiting the cardiotoxic effect of daunorubicin in rats

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Abstract

The aim was to evaluate the role of the combination of olmesartan, an angiotensin II (Ang II) receptor blocker (ARB), with daunorubicin (DNR) in reducing cardiac toxicity in rats. DNR was administered at a dose of 3 mg/kg/day every other day for 12 days. Olmesartan was administered orally every day for 12 days. Rats treated with DNR alone showed cardiac toxicity as evidenced by worsening cardiac function, elevation of malondialdehyde level in heart tissue and decreased in the level of total glutathione peroxidase activity; treatment with ARB reversed these changes. Furthermore, ARB treatment down-regulated matrix metalloproteinase-2 expression, myocardial expression of Ang II, attenuated the increased protein expressions of $p67^{phox}$ and Nox4 and reduced oxidative stress-induced DNA damage evaluated by expression of 8-hydroxydeoxyguanosine. In conclusion, the result demonstrated that Ang II and oxidative stress play a key role in anthracycline-induced cardiotoxicity and that treatment with ARB will be beneficial against DNR-induced cardiotoxicity.

Keywords: *Daunorubicin, cardiotoxicity, angiotensin II receptor blocker, oxidative stress, olmesartan.*

Introduction

The clinical use of anthracyclines (daunorubicin (DNR), doxorubicin (DOX), epirubicin, idarubicin, etc.) has been hindered by a form of cardiac toxicity that appears to be unique to this drug family [1]. Several approaches have been considered in this respect: (i) the development of anthracycline analogues which are devoid of cardiac toxicity; (ii) the use of alternative schedules of administration such as protracted infusions; (iii) the encapsulation of the anti-cancer drug in liposomal or other particulate formations; and (iv) the combination of anthracycline with a cardioprotector such as dexrazozane which has had its efficacy proven in the clinical setting [2].

In spite of extensive investigation, the mechanism of cardiac toxicity of anthracycline has not yet been completely elucidated. Several hypotheses have been proposed to explain anthracycline-induced cardiac toxicity, including free radical formation, myocyte apoptosis, lipid peroxidation, mitochondrial impairment, alteration in calcium handling and direct suppression of muscle-specific gene expression [3].

The renin-angiotensin system (RAS) is a central component of the physiological and pathological responses of the cardiovascular system. Its primary effector hormone, angiotensin II (Ang II), not only mediates immediate physiological effects of vasoconstriction and blood pressure regulation, but is also

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implicated in inflammation, endothelial dysfunction, atherosclerosis, hypertension and congestive heart failure. Ang II type-1 receptor (AT-1R)-mediated NADPH oxidase activation leads to generation of reactive oxygen species (ROS), which have been widely implicated in vascular inflammation and fibrosis [4]. Previously, we have reported that candersartan, an angiotensin receptor blocker (ARB), significantly improved the left ventricular (LV) function and reversed the myocardial pathological changes in DNR-induced cardiomyopathy [5]. In addition, RNH-6270, an active metabolite of olmesartan medoxomil (CS-866) [6], was shown to reduce DOX-induced cardiomyopathy in mice [7]. In studies on humans, valsartan can inhibit acute cardiotoxicity after DOX-based chemotherapy [8]. These results suggest that the Ang II plays a key role in the process of anthracycline-induced cardiotoxicity.

The above study [7] concluded that the AT1-mediated Ang II signalling pathways play an important role in DOX-induced cardiac impairment, suggesting that an AT1 antagonist can be used to prevent-DOX induced cardiomyopathy; however, no detailed observation has been performed on oxidative damage by ROS, associated with Ang II and AT-1R expression in DNR-induced cardiotoxicity. In the present study, we investigated the changes in myocardial function and sarcomeric protein (cardiac troponin I- cTnI) [9], as well as expression of Ang II and AT-1R in the myocardial tissue and oxidative stress-related factors in rats administered DNR, as well as in rats given DNR in combination with olmesartan.

Materials and methods

Drugs and chemicals

Unless otherwise stated all reagents were of analytical grade and were purchased from Sigma (Tokyo, Japan). DNR was kindly donated by Meiji Seika Kaisha Ltd (Tokyo, Japan). Olmesartan was donated by Daichi-Sankyo Pharmaceutical (Tokyo, Japan).

Experimental animals

Male Sprague-Dawley rats (10–12 weeks old) weighing 350–400 g were purchased from Charles River Japan Inc. (Kanagawa, Japan). DNR (diluted with 0.9% NaCl) was administered at 3 mg/kg/day i.p., every other day for 12 days (18 mg/kg total dose). The dose and schedules were chosen on the basis of our preliminary study and a previous report [10]. Twenty DNR-treated rats were randomly divided into two groups and received oral administration of olmesartan (10 mg/kg/day; group DNR+Olm; $n = 10$) or vehicle (group DNR; $n = 10$). The dose of olmesartan was chosen on the basis of the previous reports [11,12]. Age-matched rats were injected with corresponding volumes of 0.9% NaCl and used as a control (group N; $n = 5$). Olmesartan was administered orally by gavage every day for 12 days. The rats were weighed every day and assessed for possible abnormalities (ascites, bleeding, diarrhoea, etc.). Throughout the study, all animals were cared for in accordance with the guidelines of our institute and the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health.

Haemodynamic and echocardiographic study

Rats were anaesthetized with 2% halothane in O_2 and subjected to surgical procedures to measure haemodynamic parameters on day 13. After the instrumentation, the concentration of halothane was reduced to 0.5% to record steady-state haemodynamic data. Haemodynamic parameters such as mean blood pressure (MBP), peak LV pressure (LVP), LV end-diastolic pressure (LVEDP) and the rate of intraventricular pressure rise and decline ($\pm dP/dt$) were recorded as previously described [13].

Two-dimensional echocardiographic studies were performed under 0.5% halothane anaesthesia using an echocardiographic machine equipped with a 7.5-MHz transducer (SSD-5500; Aloka, Tokyo, Japan). M-mode tracings were recorded from the epicardial surface of the right ventricle; the short axis view of the left ventricle was recorded to measure the LV dimension in diastole (LVDd) and LV dimension in systole (LVDs). LV fractional shortening (FS) and ejection fraction (EF) were calculated and expressed as percentages. The study was performed in a blinded manner.

Histopathological studies

The LV portion of heart tissues was fixed in 10% neutral buffered formalin. Sections of 3–5 μm thickness were stained with haematoxylin and eosin (HE) for histological examination. A histomorphological evaluation of all the heart sections was carried out in a blinded fashion by a pathologist who was unaware of the treatment groups.

Protein analysis by western blotting

Protein lysate was prepared from heart tissue as described previously [14]. The total protein concentration in samples was measured by the bicinchoninic acid method [15]. For the determination of protein levels of cTnI, matrix metalloproteinase-2 (MMP-2), AT-1R and NADPH oxidase sub-units $p67^{phox}$ and Nox4, equal amounts of protein extracts (30 μg) were separated by sodium dodecyl sulphate (SDS)

polyacrylamide gel electrophoresis (Bio-Rad, CA) and transferred electrophoretically to nitrocellulose membranes. The concentrations of SDS were 12.5% for cTnI, 10% for AT-1R, $p67^{phox}$ and Nox4 and 7.5% for MMP-2. Membranes were blocked with 5% non-fat dry milk in Tris-buffered saline Tween (20 mM Tris, pH 7.6, 137 mM NaCl and 0.1% Tween 20). All antibodies were purchased from Santa Cruz Biotechnology Inc. (CA) and used at a dilution of 1:1000. The membrane was incubated overnight at 4°C with the primary antibody and the bound antibody was visualized using the respective horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology Inc.) and chemiluminescence developing agents (Amersham Biosciences, Buckinghamshire, UK). The level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was estimated in every sample to check for equal loading of samples. Films were scanned and band densities were quantified with densitometric analysis using the Scion Image program (Epson GT-X700, Tokyo, Japan). All values were normalized by setting the density of normal samples as 1.0.

Immunohistochemical assay

Formalin-fixed, paraffin-embedded cardiac tissue sections were used for immunohistochemical staining. After deparaffinization and hydration, the slides were washed in Tris-buffered saline (TBS; 10 mM/l Tris HCl, 0.85% NaCl, pH 7.2). Endogenous peroxidase activity was quenched by incubating the slides in methanol and 0.3% H_2O_2 in methanol. After overnight incubation with the primary antibody, either rabbit polyclonal anti-Ang II (Peninsula Laboratories Inc., San Carlos, CA) or mouse monoclonoal anti-8-hydroxydeoxyguanosine (8-OHdG) (diluted 1:100) (Abcam, Cambridge, MA), at 4°C, the slides were washed in TBS and then HRP-conjugated secondary antibody was then added and the slides were further incubated at room temperature for 45 min. The slides were washed in TBS and incubated with diaminobenzidine tetrahydrochloride as the substrate and counterstained with haematoxylin. A negative control without primary antibody was included in the experiment to verify the antibody specificity. Brown coloured immunopositive Ang II spots were counted at 400-fold magnification. The intensity of 8-OHdG expression in the myocardium was measured at 400-fold magnification in the section using a range where 0 was no labelling, 1 was $< 50\%$ area labelling of low intensity, 2 was $> 50\%$ area labelling with low intensity or $< 50\%$ area labelling with high intensity and 3 was $> 50\%$ area labelling of high intensity as described previously [16] and recorded as an index of expression. For both cases, 10 random fields representing the whole section were examined per section and three animals were used per group.

Measurement of malondialdehyde (MDA) content

Heart tissues were rinsed, weighed, resuspended at 50 mg/ml in normal saline and homogenized. After centrifugation at 5000 rpm for 10 min at 4°C, the supernatants were collected and analysed with

Table I. Changes in histopathology, haemodynamic and echocardiographic parameters after DNR treatment alone and in combination with olmesartan (10 mg/kg/day).

	Group N ($n = 5$)	Group DNR ($n = 10$)	Group DNR + Olm ($n = 10$)
Number of animal deaths	0	4	2
Histopathology			
BW (g)	380 ± 5.7	237 ± 9.6*	254 ± 7.9*
HW (g)	0.94 ± 0.02	0.69 ± 0.04*	0.66 ± 0.03*
HW/BW (g/kg)	2.52 ± 0.04	2.74 ± 0.18*	2.7 ± 0.08*
Haemodynamic and echocardiographic parameters			
MBP (mmHg)	97 ± 3.7	68.6 ± 10.3*	59.5 ± 9.1*
LVP (mmHg)	125.5 ± 5.7	96 ± 6.9*	94.7 ± 5.5*
LVEDP (mmHg)	6 ± 0.9	11 ± 1.2*	7.2 ± 1.2#
$+dP/dt$ (mmHg/s)	6580 ± 246	4722 ± 295*	6118 ± 113 #
$-dP/dt$ (mmHg/s)	7394 ± 603	4327 ± 379*	7009 ± 160 #
HR (beats/min)	411 ± 16	296 ± 1.7	301 ± 55
LVDd (mm)	6.5 ± 0.45	7.35 ± 0.35*	6.26 ± 0.67#
LVDs (mm)	3.25 ± 0.41	5.52 ± 0.57*	4.1 ± 0.55#
FS (%)	47.5 ± 3.24	22.3 ± 3.84*	35.84 ± 2.7#
EF (%)	83.4 ± 3.47	49.44 ± 7.02*	7 0.96 ± 3.67#

Results are presented as the mean ± SEM. BW, body weight; HW, heart weight; HW/BW, ratio of heart weight to body weight; MBP, mean blood pressure; LVP, left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; $\pm dP/dt$, rate of intra-ventricular pressure rise and decline; HR, heart rate; LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; FS, fractional shortening; EF, ejection fraction; group N, aged-matched normal rat; group DNR, DNR-treated rats administered with vehicle; Group DNR + Olm, DNR-treated rats administered with olmesartan (10 mg/kg/day). *$p < 0.05$ vs group N; #$p < 0.05$ vs group DNR.

corresponding assay kits (OXItex, ZeptoMetrix Corporation, New York) in accordance with the manufacturer's instructions.

Measurement of gluthathione peroxidase (GPx) activity

Heart samples were homogenized in six volumes (per wet weight of tissues) of cold GPx Assay Buffer and the mixture was centrifuged for 15 min at 4°C and 8000 rpm in accordance with the total GPx assay kit instructions (OXItek, ZeptoMetrix Corporation, New York). GPx activity in heart tissues was measured using a kinetic ultraviolet-visible spectrophotometer (Ultraspec 3100, Amersham Biosciences). The oxidation of NADPH to $NADP^+$ was measured by the decrease in absorbance at 340 nm.

Statistical analysis

Data are presented as mean ± SEM and were analysed using one-way analysis of variance (ANOVA) followed by Tukey or Bonferroni methods for post-hoc analysis and two-tailed *t*-test when appropriate. A value of $p < 0.05$ was considered statistically significant. For statistical analysis GraphPad Prism 5 software (San Diego, CA) was used.

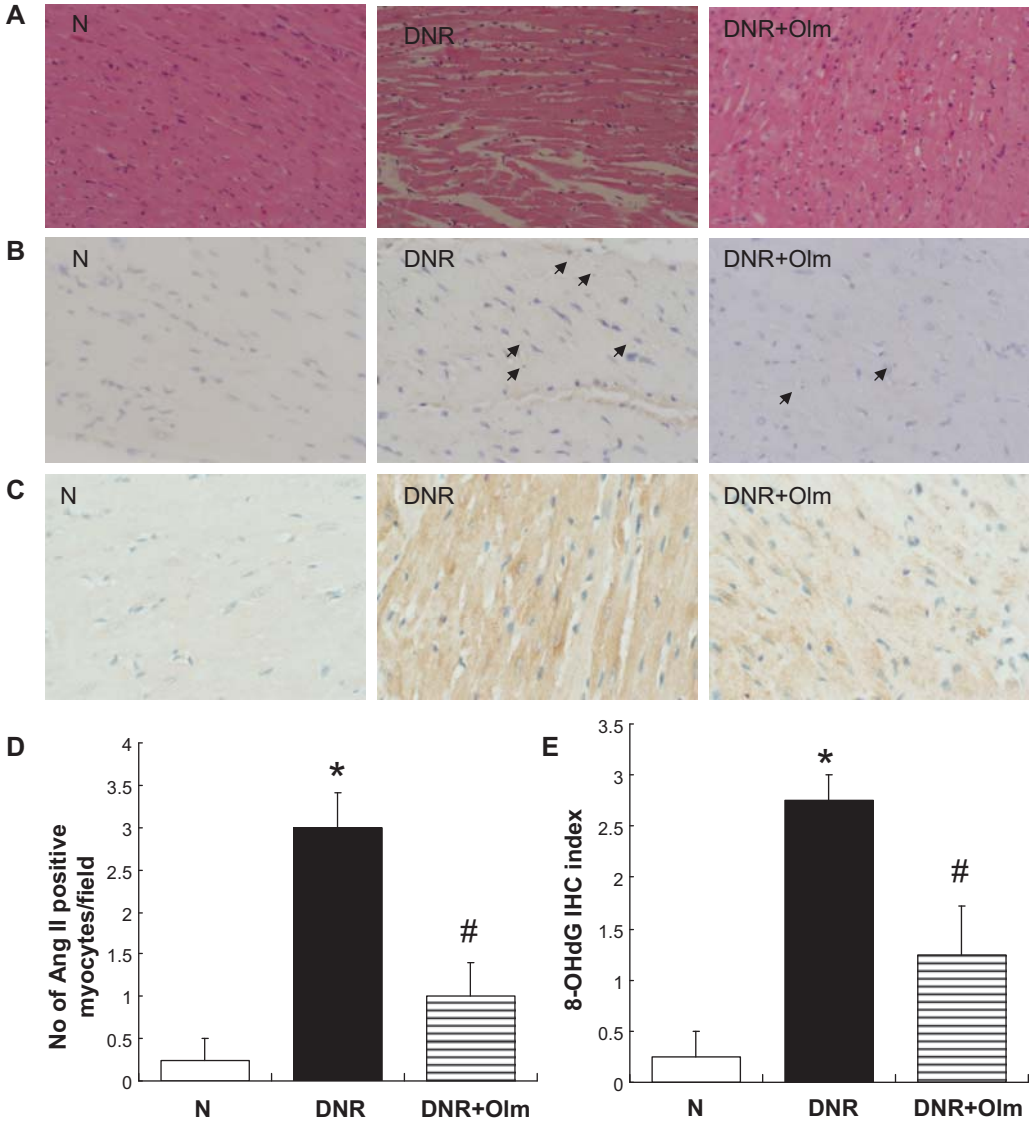

Figure 1. (A) Histopathological examination of rat heart (HE stain, 200×). Normal rat (N) shows normal morphology. DNR-treated rat (DNR) shows oedema, haemorrhage and congestions. In combination with olmesartan (DNR+Olm), the tissue appears nearly normal with mild necrosis and haemorrhage. (B) Myocardial levels of Ang II by immunohistochemical staining and the positive immunostaining of Ang II exhibit brown colour and highlighted by arrows (400×). (C) Photomicrograph of LV tissue sections showing 8-OHdG staining from groups N, DNR and DNR+Olm. 8-OHdG positive staining is shown in brown. All nuclei stained by haematoxylin are shown in blue (400×). (D) Quantification of Ang II positive myocytes per field at 400× magnification. (E) Immunohistochemistry results for 8-OHdG index of each group. Each bar represents mean ± SEM. Group N, age-matched normal rats; group DNR, DNR treated rats administered with vehicle; group DNR+Olm, DNR treated rats administered with olmesartan (10 mg/kg/day). $^*p < 0.05$ vs group N; $^\#p < 0.05$ vs group DNR.

Results

General toxicity

DNR exerted a marked general toxicity at the dose used in this experiment. In the control group, no physiological alteration was observed and the rat weight was increased by 22.2% during the 12 days of the study. Weight loss and spontaneous bleeding occurred in DNR rats with high frequency. No significant reduction in general toxicity was observed in DNR rats in combination with olmesartan (data not shown).

Histopathology, cardiac functions and protein expressions of cTnI and MMP-2

Histological analysis revealed that the heart weight (HW) and heart weight-to-body weight ratio (HW/BW, an index of hypertrophy) were significantly decreased and increased, respectively, in group DNR compared with those in group N. However, co-treatment with olmesartan did not change these parameters (Table I).

DNR-induced morphological changes in cardiac tissues were observed by light microscopy, as shown in Figure 1A. Normal morphology of the tissue was seen in group N. On the other hand, there were morphological changes in rats administered with DNR. Cross-sections of cardiac tissue of the rats from group DNR showed oedema, haemorrhage, necrosis and congestions. Meanwhile, myocardium of DNR rats treated olmesartan appeared to nearly recover to normal (Figure 1A).

Although heart rate did not differ among the three groups of rats, LVEDP was significantly higher and MBP, LVP and $\pm dP/dt$ were significantly lower in group DNR than in group N, indicating LV dysfunction in group DNR. Co-treatment with olmesartan reduced LVEDP (11 ± 1.2 mmHg vs 7.2 ± 1.2 mmHg, $p < 0.05$) and improved $\pm dP/dt$ (4722 ± 295 mmHg/s vs 6118 ± 113 mmHg/s, $p < 0.05$; and 4372 ± 379 mmHg/s vs 7009 ± 160 mmHg/s, $p < 0.05$) significantly in comparison with those in group DNR (Table I).

Echocardiographic data revealed that both LVDd and LVDs were significantly increased in group DNR compared with those in group N (6.5 ± 0.45 mm vs 7.35 ± 0.35 mm, $p < 0.05$; and 3.25 ± 0.41 mm vs 5.52 ± 0.57 mm, $p < 0.05$; respectively). In addition, LV systolic function, as assessed by FS and EF, was also reduced in group DNR compared with that in group N (47.5 ± 3.24% vs 22.3 ± 3.84%, $p < 0.05$; and 83.4 ± 3.47% vs 49.44 ± 7.02%, $p < 0.05$; respectively). Co-treatment with olmesartan significantly reduced both LVDd and LVDs and increased both FS and EF significantly compared with those in group DNR (Table I).

Significant decreases in the protein expression of cTnI and increases in the protein expression of MMP-2 were found in group DNR compared with those in group N. Co-treatment with olmesartan attenuated the decreases in cTnI and increases in MMP-2 protein expression (Figures 2A–C).

Myocardial level of Ang II, AT-1R and oxidative stress

Myocardial immunoreactivity for Ang II was little or absent in the heart of group N. Heart sections of group DNR showed stronger immunoreactivity for Ang II than those of group N (Figures 1B and D) and immunohistchemical analysis of group DNR+Olm revealed a significant decrease in the myocardial level of Ang II (Figures 1B and D).

Significant increases in MDA level and decreases in total GPx activity in the heart tissue were found in group DNR in comparison with those in group N. Co-treatment with olmesartan significantly decreased

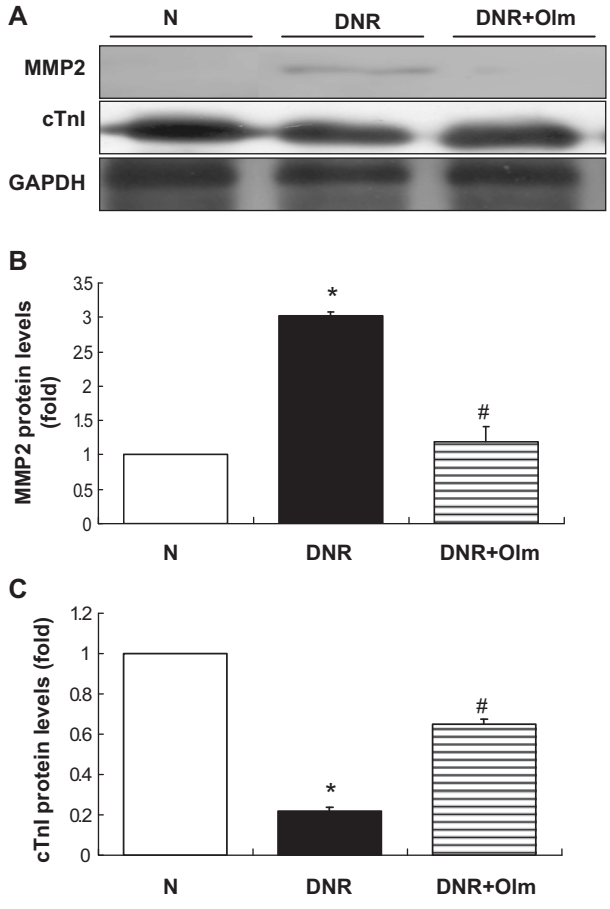

Figure 2. Myocardial expressions of MMP2 and cTnI. (A) Representative Western blots showing specific bands for MMP2, cTnI and GAPDH as an internal control. Equal amounts of protein sample obtained from whole ventricular homogenate were applied in each lane. These bands are representative of five separate experiments. (B) and (C) Densitometric data of protein analysis. The mean density values of MMP2 and cTnI were expressed as ratios relative, to that of group N. Each bar represents mean ± SEM. Group N, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group DNR+Olm, DNR-treated rats administered with olmesartan (10 mg/kg/day). $^{*}p < 0.05$ vs group N; $^{\#}p < 0.05$ vs group DNR.

MDA level and increased GPx activity compared with those in group DNR (Figures 3A and B).

The protein levels of NADPH oxidase sub-units p67^{phox} and Nox4 as well as AT-1R levels were significantly increased in group DNR compared with those in group N and these increases were significantly reduced in group DNR+Olm (Figures 4A–D). In addition, myocardial expression of 8-OHdG was significantly increased in DNR-treated rat, compared with that in group N and the increase was attenuated by the co-treatment with olmesartan (Figures 1C and E).

Discussion

The results of the present study demonstrated that co-treatment DNR with oral olmesartan improved both systolic (+dP/dt, % EF and % FS) and diastolic variables (-d*P*/d*t* and LVEDP) and caused an improvement in cardiac histological damage as well as decrease in the level of oxidative stress marker. These results suggested that olmesartan showed a cardioprotective effect in a rat model of anthracyclines-mediated toxicity.

Anthracycline has become widely used in the treatment of haematological malignancies and solid tumours, but its cumulative toxicity on the myocardium prevents its use at a maximum myelotoxic dose for the optimal number of therapeutic cycles required [17]. Heart damage due to anthracycline therapy is a considerable and serious problem. Three distinct types of anthracycline-associated cardiotoxicity (acute, chronic and late onset) have been reported [18]; therefore, it is important to reduce or prevent anthracycline-induced cardiotoxicity.

This study has confirmed that a cumulative dose of DNR (18 mg/kg ip) induces acute cardiotoxicity in rats, which is in agreement with the previous studies [10,19]. In this study, MDA levels were significantly elevated after DNR treatment which supported the hypothesis that free radicals play a major role in anthracycline toxicity [20]. The reduction in the levels of MDA in the heart tissue of olmesartan-treated rats suggests that it protects myocardium against DNR-induced lipid peroxidation. In addition, in this study, the activity of the GPx enzyme (an important enzyme against oxidative stress) decreased in DNR-rats and the combination with ARB resulted in recovery from depletion of this enzyme. These findings were consistent with other reports [21–24]. In line with our results, Ibrahim et al. [25] showed that telmisartan and captopril have equally protective effects against DOX-induced cardiotoxicity, on the basis of their antioxidant effects.

DNR-induced morphological changes in the cardiac tissue were observed by light microscopy. Recent studies have reported that anthracycline treatment induces significant morphological damage and these changes were attenuated by treatment with an ARB or iron chelator [23,26]. Consistent with the previous reports, we have also observed morphological changes in the cardiac tissue of the DNR group and as a consequence of the above biochemical changes, the LV myocardial damage was also less pronounced in the group treated with olmesartan.

It has been reported that Ang II plays an important role in DOX-induced cardiac injury [7,23,25] and, in the present study, this was confirmed by an increase in the number of Ang II-positive cardiomyocytes (Figures 1B and D) and the myocardial expression of AT-1R (Figures 4A and B) in the rats of the DNR group and the fact that the combination of DNR with ARB attenuated the increased expression levels of Ang II and AT-1R in heart tissue. Moreover, previous clinical and experimental studies showed that Ang II induces oxidative damage by producing ROS through the NADH/NADPH oxidase system [27,28]. To address the role of ROS, we measured the expression levels of NADPH sub-units: p67^{phox} and Nox4 in heart tissues by western blotting. Our results showed that DNR increased the expression levels of NADPH oxidase sub-units significantly and that these effects were inhibited by the co-treatment with olmesartan. In addition, the level of myocardial 8-OHdG, which is a sensitive and specific marker of DNA damage induced by oxidative stress [29], was significantly increased in the DNR group. In contrast, myocardial

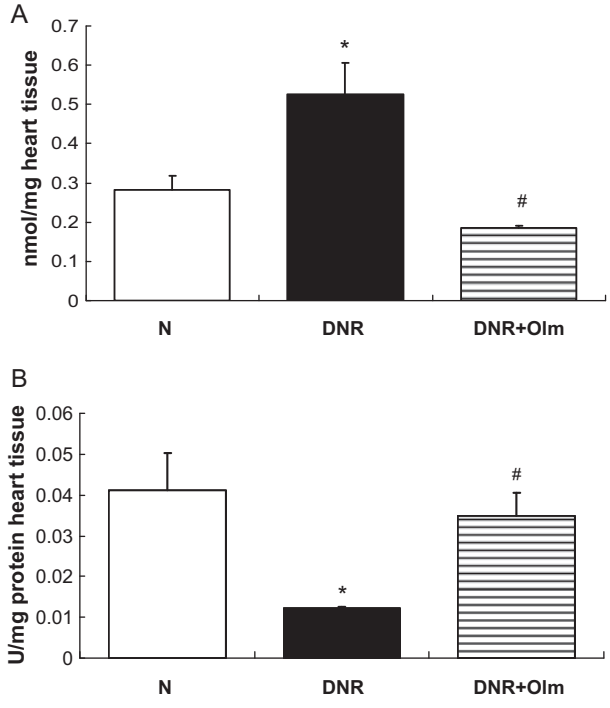

Figure 3. Effects of ARB on lipid peroxidation by measuring MDA levels (A) and GPx activity (B) in heart homogenates from DNR-treated rats. The values are means ± SEM. Group N, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group DNR+Olm, DNR-treated rats administered with olmesartan (10 mg/kg/day). $^{*}p < 0.05$ vs group N; $^{\#}p < 0.05$ vs group DNR.

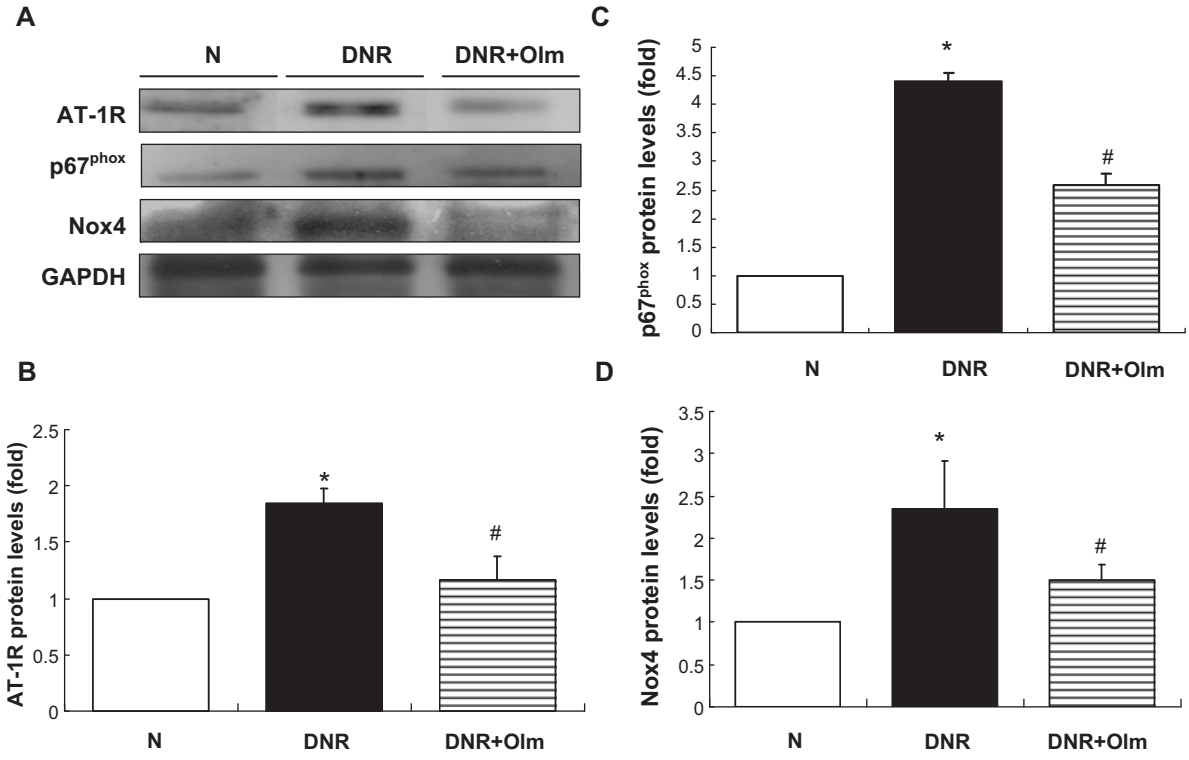

Figure 4. Myocardial expressions of AT-1R, $p67^{phox}$, Nox4 and GAPDH. (A) Representative Western blots showing specific bands for AT-1R, $p67^{phox}$, Nox4 and GAPDH as an internal control. Equal amounts of protein sample obtained from whole ventricular homogenate were applied in each lane. These bands are representative of five separate experiments. (B–D) Densitometric data of protein analysis. The mean density values of AT-1R, $p67^{phox}$ and Nox4 were expressed as ratios relative to that of group N. Each bar represents mean ± SEM. Group N, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group DNR+Olm, DNR-treated rats administered with olmesartan (10 mg/kg/day). $^{*}p < 0.05$ vs group N; $^{\#}p < 0.05$ vs group DNR.

8-OHdG was decreased by the combination with olmesartan. These results suggested that the beneficial effects of ARB in anthracycline-induced cardiotoxicity might be mediated through direct inhibition of Ang II via AT-1R, which further reduces the oxidative stress.

Clinically, patients receiving anthracycline are continuously monitored by echocardiography during and after treatment and FS and EF are considered as important indicators of systolic function [30]. In addition, cardiac troponin is a sensitive biomarkers for the detection of cardiac toxicity in anthracycline treatment [9] and cTnI is important for the structural integrity and function of cardiomyocytes and its myocardial expression is reportedly down-regulated by anthracycline [31,32]. Additionally, Cardinale et al. [33] reported that cTnI was a strong predictor of cardiological outcome in cancer patients receiving high dose chemotherapy and early treatment with enalapril seems to prevent the development of late cardiotoxicity. In our study, DNR treatment resulted in the deterioration of cardiac function as indicated by the deteriorated echocardiographic parameters and down-regulated myocardial expression of cTnI. It is important to note that ARB treatment in this study has significantly improved myocardial function, as evidenced by increases of FS and EF and restoration of the expression of cTnI in the presence of DNR.

Moreover, recent studies indicate that increased MMPs activities are associated with a number of cardiac pathological changes and that MMP-2 is likely to be responsible for the proteolysis of cTnI [34]. Previous studies showed that the myocardial expression of MMP-2 is up-regulated by anthracycline [35,36], an effect that we confirmed in the present study; in addition, this increase was normalized by ARB treatment. Ang II plays a pivotal role in the synthesis of MMPs and it has been shown that there is an improvement in LV function and myocardial geometry through reduced MMP synthesis after ACE-inhibition or AT-1R blockade [37] and our results are in line with those of this previous report [37].

In conclusion, the present study provided evidence that oxidative stress via Ang II may play an essential role in DNR-induced cardiotoxicity, and that ARB treatment could improve cardiac function by reversing oxidative stress, suggesting a beneficial effect of ARB treatment in preventing anthracycline-induced cardiotoxicity. Since dexrazoxane (ICRF-187) is the only clinically approved cardioprotectant against anthracycline cardiotoxicity [38], it is of interest to combine ARB or angiotensin-converting enzyme inhibitor with dexrazoxane to evaluate the possible potential synergistic or additive effect in preventing anthracycline-induced cardiotoxicity. Further investigation is warranted to address these issues.

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