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Research Paper

Effect of telmisartan in limiting the cardiotoxic effect of daunorubicin in rats

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Abstract

Objectives Studies have suggested that angiotensin receptor blockers may exert a protective role towards doxorubicin-induced cardiotoxicity, but they have not been extensively investigated in this area. We therefore investigated whether the co-treatment of telmisartan, an angiotensin (Ang II) type-1 receptor blocker, might offer protection against daunorubicin cardiotoxic properties in rats.

Methods Daunorubicin was administered at 3 mg/kg/day every other day for 12 days. Telmisartan was administered orally every day for 12 days.

Key findings Daunorubicin-treated rats showed cardiac toxicity, evidenced by worsening cardiac function, evaluated by haemodynamic status and echocardiography, elevation of malondialdehyde level and a decreased level of total glutathione peroxidase activity in the heart tissue. These changes were reversed by treatment with telmisartan. Furthermore, telmisartan also downregulated matrix metalloproteinase-2 expression, attenuated the increased protein expression of $p22^{phox}$, $p47^{phox}$, $p67^{phox}$, nuclear factor kappa B and Nox4 in heart tissue, and reduced oxidative-stress-induced DNA damage, which was evaluated by the expression of 8-hydroxydeoxyguanosine. Moreover, telmisartan reduced the myocardial apoptosis induced by daunorubicin.

Conclusions The present study indicates that telmisartan may improve cardiac function by inhibiting the action of Ang II via AT-1R, which reverses oxidative stress and myocardial apoptosis. This suggests a beneficial effect of telmisartan treatment in the prevention of daunorubicin-induced cardiotoxicity.

Keywords angiotensin II; cardiotoxicity; daunorubicin; oxidative stress; telmisartan

Introduction

For more than 20 years, anthracycline antineoplastic drugs (daunorubicin (DNR), doxorubicin (DOX), epirubicin and idarubicin) have been among the most effective and widely used anticancer chemotherapeutics in clinical practice. Their clinical utility is, however, largely limited by the adverse reactions that accompany their use. Besides the reversible and often easily manageable adverse effects typical of anticancer drugs (e.g. nausea, myelosuppression), there is a well-documented risk of a severe complication: cardiotoxicity.[1]

Clinically, there are two types of cardiotoxicity. One type occurs rapidly after the administration of a single dose of anthracycline and is characterised by electrocardiographic changes, arrhythmias and a reversible decrease in ventricular contractile function.[2] The other type of cardiotoxicity is characterised by irreversible deterioration of ventricular contractile function, leading to congestive heart failure, and is directly related to the cumulative anthracycline dose.[3,4] Several strategies for reducing toxicity have been proposed, including dose-reduction protocols,[5] encapsulating the drug with liposomes[6] or a lipidic nanoemulsion,[7] and administration with antioxidant or metal chelators.[8]

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However, the protection afforded by these treatments has not been demonstrated to be consistently effective.[9]

In spite of extensive investigation, the mechanism of cardiac toxicity of anthracycline has yet to be 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.[10]

The renin-angiotensin system (RAS) is a central component of the physiological and pathological responses of the cardiovascular system. The activity of angiotensin II (Ang II), the main effector of RAS, is initiated by its interaction with at least two pharmacologically distinct subtypes of cell-surface receptor, AT-1 and AT-2. The major functions of Ang II in the cardiovascular system are mediated by the AT-1 receptor (AT-1R).[11] Recent findings have suggested that Ang II activates intracellular signalling processes, which leads to events that include the generation of reactive oxygen species (ROS), myocardial apoptosis and fibrosis.[12]

Previous studies have reported that Ang II plays a key role in the process of anthracycline-induced cardiotoxicity.[13,14] However, studies have suggested that angiotensin-converting enzyme (ACE) inhibitor and angiotensin receptor blocker (ARB) exert a protective role towards DOX-induced cardiotoxicity,[14–16] but this effect has not been investigated in DNR-induced cardiotoxicity. Furthermore, the exact mechanism of action and efficacy of ARB in DNR-induced cardiotoxicity are not clear, therefore further experimental studies are needed in order to verify its efficacy and elucidate its exact mechanism of action.

Recently, some investigators have shown that telmisartan, an ARB, is a partial agonist of the peroxisome proliferative activated receptor-gamma (PPAR-γ), and it is reported to possess anti-inflammatory and antioxidant properties.[17–19] To the best of our knowledge, no published study has investigated the cardioprotective effects of telmisartan in DNR-induced toxicity. In the present study, we have therefore investigated the mechanism of its protective effect against DNR-induced cardiotoxicity in rats, using changes in myocardial function, histopathology, myocardial apoptosis, and biochemical and oxidative-stress-related factors.

Materials and Methods

Drugs and chemicals

Unless otherwise stated, all reagents were of analytical grade and purchased from Sigma (Tokyo, Japan). DNR was kindly donated by Meiji Seika Kaisha Ltd (Tokyo, Japan). Telmisartan was generously provided by Boehringer Ingelheim GmbH (Ingelheim am Rhein, Germany).

Experimental animals and protocol

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 i.p. at 3 mg/kg/day every other day for 12 days (18 mg/kg total dose). The dose of DNR used was determined on the basis of an earlier study.[20] Twenty DNR-treated rats were randomly divided into two groups and orally administered telmisartan (10 mg/kg/day; group Telm; $n = 10$) or vehicle (group DNR; $n = 10$). Age-matched rats were injected with corresponding volumes of 0.9% NaCl and used as a control (group N; $n = 5$). Telmisartan 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.). The animal experiments were performed in accordance with national guidelines for the use and care of laboratory animals and were approved by the local animal care committee of Niigata University of Pharmacy and Applied Life Sciences.

Haemodynamic and echocardiographic study

Rats were anaesthetised 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 such as left ventricular (LV) end-diastolic pressure (LVEDP), and the rate of intra-ventricular pressure rise and decline ($\pm dP/dt$).

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 the LV dimension in systole (LVDs). LV fractional shortening (FS) and ejection fraction (EF) were calculated and expressed as percentages. The study was performed blind.

Biochemical studies

The thiobarbituric acid reactive substance (TBARS) assay was used to measure lipid peroxidation. TBARS are expressed in terms of malondialdehyde (MDA) equivalents. The heart-tissue content of MDA was determined using TBARS assay kits (OXItex, ZeptoMetrix Corporation, New York, USA) in accordance with the manufacturer's instructions.

For gluthathione peroxidase (GPx) activity, heart samples were homogenised in six volumes (per wet weight of tissue) of cold GPx assay buffer, and the mixtures were centrifuged for 15 min at 4°C and 8000 rpm in accordance with the instructions of the total GPx assay kit (OXItek, ZeptoMetrix Corporation, New York, USA). The oxidation of NADPH to $NADP^+$, which is indicative of GPx activity, was measured by the decrease in absorbance at 340 nm, using a kinetic ultraviolet-visible spectrophotometer (Ultraspec 3100, Amersham Biosciences).

Histopathological studies

Rat heart tissue was removed immediately after myocardial functional analyses, washed with normal saline and 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 (H&E) for histological examination. A histomorphological evaluation of all the heart sections was carried out blind by a pathologist.

Immunohistochemical assay

Formalin-fixed, paraffin-embedded cardiac tissue sections were used for immunohistochemical staining. After deparaffinisation and hydration, the slides were washed in Tris-buffered saline (TBS; 10 mM 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, namely anti-8-hydroxydeoxyguanosine (8-OHdG) antibody (diluted 1 : 1000) (Abcam, Cambridge, MA) at 4°C, the slides were washed in TBS and horseradish peroxidase (HRP)-conjugated rabbit anti-mouse 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. The area and intensity of staining were scored under light microscopy. The intensity of 8-OHdG expression in the myocardium was measured at ×400 magnification in the section using a scale where 0 represented no labelling; 1, <50% of the area labelled at low intensity; 2, >50% of the area labelled at low intensity or <50% of the area labelled at high intensity; and 3, >50% of the area labelled at high intensity. These scorings are as described previously.[21] Four sections per animal and 10 fields per section were analysed. The averages were calculated for each experimental group and recorded as an index of expression.

In-situ terminal deoxynucleotidyl transferase-mediated nick-end labelling assay

The terminal deoxynucleotidyl transferase-mediated nick-end labelling (TUNEL) assay was performed as specified in the instructions for the in-situ apoptosis detection kit (Takara Bio. Inc., Shiga, Japan) using paraffin sections. For each animal, three sections were scored for apoptotic nuclei. Only nuclei that were clearly located in cardiac myocytes were considered. For each slide 10 fields were randomly chosen, and by using a defined rectangular field area, a total of 100 cells per field were counted. The percentage of total myocytes that were TUNEL-positive (apoptotic index) was then calculated. This evaluation was performed by a person who was blind to the treatment group.

Protein analysis by Western blotting

Protein lysate was prepared from heart tissue as described previously.[22] The total protein concentration in samples was measured by the bicinchoinic acid method.[23] For determination of the protein levels of cardiac troponin I (cTnI), matrix metalloproteinase-2 (MMP-2), AT-1R, caspase-7, B-cell leukaemia/lymphoma 2 (Bcl-2), nuclear factor kappa B (NF-κB) p65 subunit, thioredoxin reductase (Trx-R), $p22^{phox}$, $p47^{phox}$, $p67^{phox}$ and Nox4, equal amounts of protein extracts (30 μg) were separated by 12.5% (for cTnI, caspase-7 and $p22^{phox}$), 10% (for AT-1R, Bcl-2, NF-κB p65, Trx-R, $p47^{phox}$, $p67^{phox}$ and Nox4) and 7.5% (for MMP-2) sodium dodecyl sulphate polyacrylamide gel electrophoresis (Bio-Rad, CA, USA) and transferred electrophoretically to nitrocellulose membranes. Membranes were blocked with 5% non-fat dry milk in TBS-T (20 mM Tris, pH 7.6, 137 mM NaCl and 0.1% Tween 20). All antibodies were purchased from Santa Cruz Biotechnology Inc. (CA, USA) and used at a dilution of 1 : 1000. After overnight incubation at 4°C with primary antibody, the bound antibody was visualised using the respective HRP-conjugated secondary antibodies (Santa Cruz Biotechnology Inc.) and chemiluminescence developing agents (Amersham Biosciences, Buckinghamshire, UK). The level of glyceraldehyde 3-phosphate dehydrogenase was estimated in every sample. Films were scanned and band densities were quantified with densitometric analysis using the Scion Image program (Epson GT-X700, Tokyo, Japan). All values were normalised by setting the density of normal samples as 1.0.

Statistical analysis

Data are presented as mean ± SEM and were analysed using one-way analysis of variance (ANOVA) followed by the 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, USA) was used.

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 body weight was increased by 22.2% during the 12 days of the study. Weight loss, diarrhoea and spontaneous bleeding occurred in DNR rats with a high frequency. No significant reduction of general toxicity was observed in DNR rats when telmisartan was also administered (data not shown).

Cardiac functions, histopathology and biochemical marker

LVEDP was significantly higher and $\pm dP/dt$ was significantly lower in the DNR group than in group N, indicating LV dysfunction in the vehicle-treated DNR rats. Co-treatment with telmisartan reduced LVEDP (11 ± 1.2 mmHg versus 7.8 ± 0.4 mmHg, $P < 0.05$) and improved $\pm dP/dt$ (4722 ± 295 mmHg/s versus 6635 ± 90 mmHg/s, $P < 0.05$; and 4327 ± 379 mmHg/s versus 6969 ± 141 mmHg/s, $P < 0.05$) significantly in comparison with those in group DNR (Table 1).

Echocardiographic data revealed that both LVDd and LVDs were significantly increased in group DNR compared with those in group N. In addition, LV systolic function, as assessed by FS and EF, was also significantly reduced in group DNR compared with group N (Table 1). The increases in both LVDd and LVDs were significantly attenuated in group Telm, since FS and EF were significantly increased (Table 1).

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 group DNR. The cross-section of cardiac tissue of the rat showed evidence

Table 1 Changes in body weight, heart rate and their ratio to each other, haemodynamic, echocardiographic and biochemical parameters after DNR treatment alone and in cotreatment with telmisartan

	Group N $n = 5$	Group DNR $n = 6$	Group Telm $n = 8$
Weights			
BW (g)	380 ± 5.7	237 ± 9.6*	244 ± 10.6*
HW (g)	0.94 ± 0.02	0.69 ± 0.04*	0.65 ± 0.03*
Ratio HW/BW (g/kg)	2.52 ± 0.04	2.74 ± 0.18*	2.75 ± 0.12*
Haemodynamic and echocardiographic parameters			
LVEDP (mmHg)	6 ± 0.9	11 ± 1.2*	7.8 ± 0.4#
+d*P*/d*t* (mmHg/s)	6580 ± 246	4722 ± 295*	6635 ± 90#
−d*P*/d*t* (mmHg/s)	7394 ± 603	4327 ± 379*	6969 ± 141#
LVDd (mm)	6.5 ± 0.45	7.35 ± 0.35*	6.87 ± 0.37#
LVDs (mm)	3.25 ± 0.41	5.52 ± 0.57*	4.62 ± 0.44#
FS (%)	47.5 ± 3.24	22.3 ± 3.84*	32.63 ± 6.3#
EF (%)	83.4 ± 3.47	49.44 ± 7.02*	64.87 ± 8.23#
Biochemical parameters			
MDA (nmol/mg)	0.28 ± 0.03	0.53 ± 0.07*	0.24 ± 0.05#
GPx activity (U/mg protein)	0.04 ± 0.01	0.01 ± 0.0004*	0.034 ± 0.008#

BW, body weight; HW, heart weight; HW/BW, ratio of heart weight to body weight; ±d*P*/d*t*, rate of intra-ventricular pressure rise and decline; LVEDP, left ventricular end-diastolic pressure; LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; FS, fractional shortening; EF, ejection fraction; MDA, malondialdehyde; GPx, gluthathione peroxidase; group N, aged-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group Telm, DNR-treated rats administered with telmisartan (10 mg/kg/day). Results are presented as the mean ± SEM.*$P < 0.05$ versus group N; #$P < 0.05$ versus group DNR.

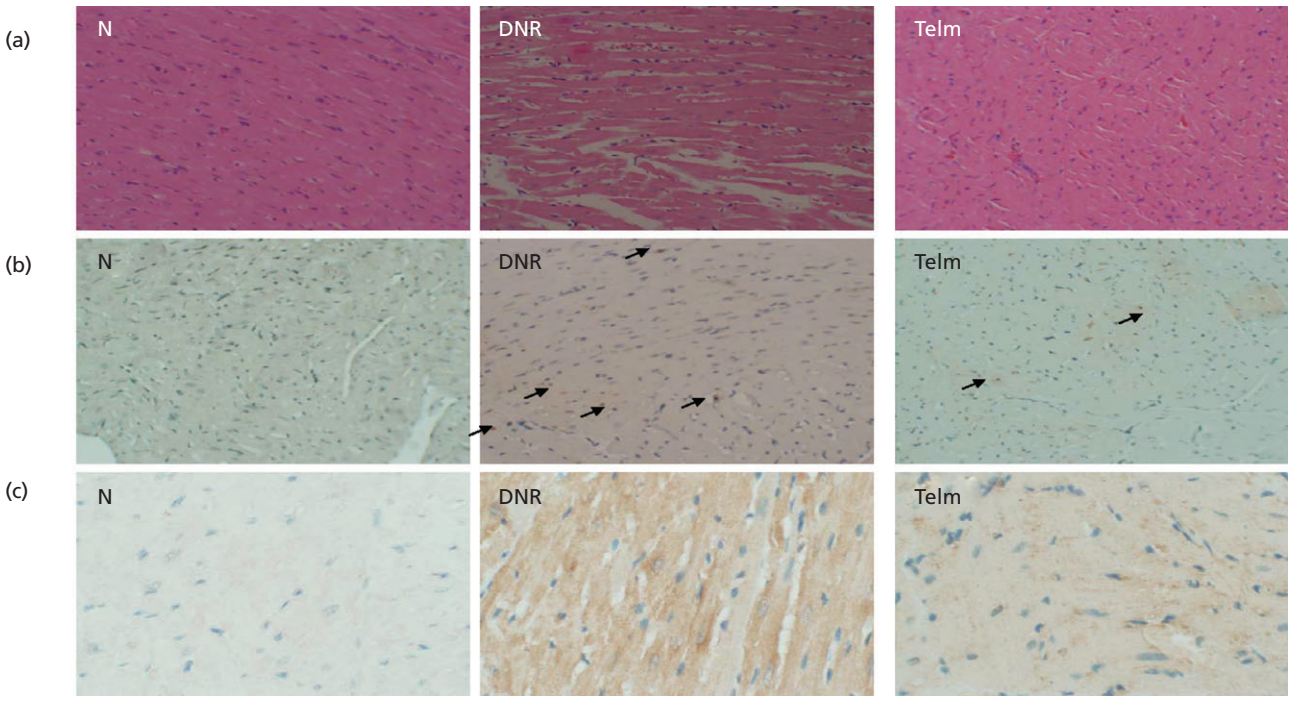

Figure 1 (a) Histopathological examination of rat heart (H&E 200X). Group N rats (N) show normal morphology. DNR-treated rats (DNR) show oedema, haemorrhage and congestions. With cotreatment with telmisartan (Telm) the tissue appears to regress to normal. (b) Myocardial tissue sections stained for TUNEL-positive apoptotic nuclei (indicated by arrows); images were taken at ×200 magnification. (c) Photomicrograph of left ventricular tissue sections showing 8-OHdG staining from groups N, DNR and Telm. 8-OHdG-positive staining is shown in brown. All nuclei stained by haematoxylin are shown in blue (×400 magnification).

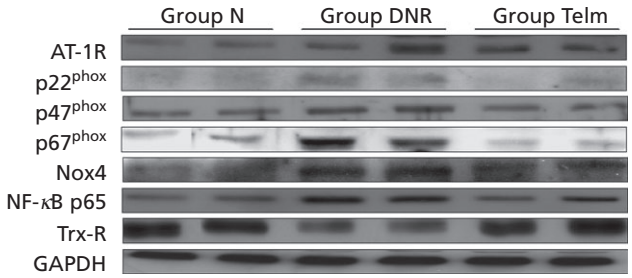

Figure 2 Western immunoblots showing the myocardial expression of AT-1R, $p22^{phox}$, $p47^{phox}$, $p67^{phox}$, Nox4, NF-κB p65, Trx-R and GAPDH as an internal control. These immunoblots are representative of five separate experiments. Equal amounts of protein sample obtained from whole ventricular homogenate were applied in each lane. Group N, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group Telm, DNR-treated rats administered with telmisartan (10 mg/kg/day).

of oedema, haemorrhage and congestion. In contrast, the myocardium of rats treated with telmisartan appeared almost normal.

Heart weight (HW) and body weight (BW) were significantly decreased in group DNR compared with group N, whereas the ratio of HW to BW (an index of hypertrophy) was significantly increased in group DNR compared with group N. However, these parameters were not changed by treatment with telmisartan (Table 1).

Significant increases in MDA level and decreases in total GPx activity in heart tissue were found in group DNR in comparison with group N. Treatment with telmisartan significantly decreased MDA levels and increased GPx activity compared with group DNR (Table 1).

Myocardial expression of AT-1R, $p22^{phox}$, $p47^{phox}$, $p67^{phox}$, Nox4, NF-κB p65 and Trx-R, and myocardial staining of 8-OHdG

The myocardial expression of AT-1R was significantly increased in group DNR compared with group N. Treatment with telmisartan significantly attenuated the increased expression of myocardial AT-1R. The protein levels of NADPH oxidase subunits $p22^{phox}$, $p47^{phox}$, $p67^{phox}$ and Nox4 were significantly increased in group DNR compared with group N, and these increases were significantly reduced in the telmisartan group (Figure 2 and Table 2). On the other hand, DNR caused a significant decrease in Trx-R protein level and an increase in NF-κB p65 protein levels compared with group N. Cotreatment with telmisartan attenuated these changes (Figure 2 and Table 2). In addition, myocardial intensities of 8-OHdG, which is a sensitive and specific marker of DNA damage induced by oxidative stress, were significantly increased in vehicle-treated DNR rats compared with group N (0.5 ± 0.2 versus 2.75 ± 0.2, $P < 0.05$) and these increases were significantly attenuated by treatment with telmisartan (2.75 ± 0.2 versus 1.5 ± 0.2, $P < 0.05$) (Figure 1c).

TUNEL analysis, myocardial expression of activated caspase-7, Bcl-2, MMP-2 and cTnI

TUNEL-positive nuclei were scarce or absent in the hearts of group N, whereas the number of TUNEL-positive nuclei was markedly increased in group DNR ($0.01 \pm 0.004\%$ versus $0.1 \pm 0.01\%$, $P < 0.05$) (Figure 1b). These results match with the increased expression of activated caspase-7 and decreased expression of Bcl-2 (Figure 3, Table 2). Treatment with telmisartan significantly decreased the number of TUNEL-positive nuclei in the myocardium (0.1 ± 0.01 versus 0.03 ± 0.004, $P < 0.05$) (Figure 1b). Moreover, the myocardial expressions of activated caspase-7 and Bcl-2 were significantly decreased and increased, respectively, in group Telm compared with group DNR (Figure 3, Table 2).

Significant decreases and increases in the protein expressions of cTnI and MMP-2, respectively, were found in group DNR compared with group N. Telmisartan significantly attenuated the decreases in cTnI and increases in MMP-2 protein expression (Figure 3, Table 2).

Table 2 Quantification of protein expressions

	Group N $n = 5$	Group DNR $n = 5$	Group Telm $n = 5$
AT-1R	1.00 ± 0.00	1.85 ± 0.13*	$1.06 \pm 0.2^{\#}$
$p22^{phox}$	1.00 ± 0.00	2.18 ± 0.12*	$1.12 \pm 0.04^{\#}$
$p47^{phox}$	1.00 ± 0.00	4.59 ± 0.48*	$3.06 \pm 0.53^{\#}$
$p67^{phox}$	1.00 ± 0.00	4.39 ± 0.17*	$2.53 \pm 0.32^{\#}$
Nox4	1.00 ± 0.00	2.34 ± 0.57*	$1.41 \pm 0.21^{\#}$
NF-κB p65	1.00 ± 0.00	2.36 ± 0.08*	$1.33 \pm 0.03^{\#}$
Trx-R	1.00 ± 0.00	0.38 ± 0.02*	$0.78 \pm 0.01^{\#}$
Bcl-2	1.00 ± 0.00	0.53 ± 0.02*	$1.09 \pm 0.03^{\#}$
Caspase-7	1.00 ± 0.00	2.02 ± 0.09*	$1.28 \pm 0.12^{\#}$
MMP-2	1.00 ± 0.00	3.02 ± 0.06*	$1.24 \pm 0.15^{\#}$
cTnI	1.00 ± 0.00	0.22 ± 0.02*	$0.62 \pm 0.05^{\#}$

The mean density values of above protein expressions were expressed as ratios relatives to that of group N. Group N, aged-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group Telm, DNR-treated rats administered with telmisartan (10 mg/kg/day). Each value represents mean ± SEM. *$P < 0.05$ versus group N; $^{\#}P < 0.05$ versus group DNR.

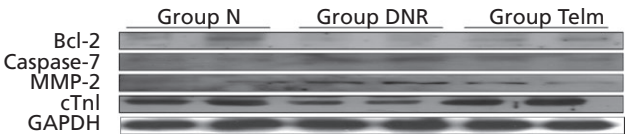

Figure 3 Western immunoblots showing the myocardial expression of Bcl-2, caspase-7, MMP-2, cTnI and GAPDH as an internal control. These immunoblots are representative of five separate experiments. Equal amounts of protein sample obtained from whole ventricular homogenate were applied in each lane. Group N, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group Telm, DNR-treated rats administered with telmisartan (10 mg/kg/day).

Discussion

The results of the present study demonstrate that administration of the combination of DNR with telmisartan reduces oxidative stress, oxidative DNA damage and TUNEL-positive nuclei in the myocardium more than the administration of DNR alone. These results are accompanied by an improvement in LV function – both systolic (+dP/dt, %EF and %FS) and diastolic variables (–dP/dt and LVEDP).

Clinically, patients receiving anthracycline are continuously monitored by echocardiography during and after treatment, and FS and EF are considered to be 'standard indicators' of LV function used for the diagnosis of cardiotoxicity.[24] In addition, cardiac troponin is a sensitive biomarker for the detection of cardiac toxicity in anthracycline treatment,[25] while cTnI is important for the structural integrity and function of cardiomyocytes and its myocardial expression is reportedly downregulated by anthracycline.[26,27] Additionally, Cardinale *et al*. reported that cTnI was a strong predictor of cardiological outcome in cancer patients receiving high-dose chemotherapy, and early treatment with enalapril appeared to prevent the development of late cardiotoxicity.[28]

Since clinical cardiac changes were difficult to interpret by echocardiography in this short-term (12 days) study, we therefore additionally measured myocardial functional parameters (LVEDP, $\pm$dP/dt) by haemodynamic analyses to further strengthen the findings on cardiac changes after DNR treatment. In our study, DNR treatment resulted in the deterioration of contractile function, as indicated by worsening haemodynamic and echocardiographic parameters, and downregulated myocardial expression of cTnI. It is important to note that telmisartan treatment significantly improved the contractile functional parameters and restored the expression of cTnI in the presence of DNR (Figure 3, Tables 1 and 2). Moreover, recent studies indicate that increased MMP activities are associated with a number of cardiac pathological changes and MMP-2 is likely to be responsible for the proteolysis of cTnI.[29] In addition, previous studies indicate that the myocardial expression of MMP-2 is upregulated by anthracycline,[30,31] an effect we confirmed in the present study. This increase was normalised by telmisartan treatment, which resulted in an improvement in myocardial function. Our results are in line with those of a previous report that stated that improvements in LV function and geometry are observed through reduced MMP synthesis after ACE inhibition or AT-1 blockade, which suggests that Ang II plays a pivotal role in the synthesis of MMPs.[32]

It was shown in our study that a cumulative dose of DNR (18 mg/kg i.p.) induces acute cardiotoxicity in rats, which is in agreement with the findings of previous studies.[20,33] DNR-induced cardiotoxicity is associated with marked elevation of the myocardial concentration of the lipid peroxidation product (MDA), which supports the hypothesis that free radicals play a role in anthracycline toxicity.[34] These elevated levels of MDA were significantly decreased in the heart tissue of DNR rats that were also treated with telmisartan, which suggests that telmisartan protects the myocardium against DNR-induced lipid peroxidation. In addition, the activity of GPx, an important enzyme that protects against oxidative stress, was also decreased in DNR-rats, and the combination of DNR with ARB counteracted the depletion of this enzyme (Table 1). These findings were consistent with those of a previous report that suggested that oxidative stress plays a role in anthracycline-induced cardiotoxicity.[15,35–39] Moreover, it has been reported that anthracycline treatment induces significant morphological changes in the heart,[37,40] something which was also observed in our study. In the present study, as the consequence of the above biochemical changes, LV myocardial damage was also less pronounced in the group treated with telmisartan (Figure 1).

It has been shown in both clinical and experimental studies that Ang II induces oxidative damage by producing ROS through the NADH/NADPH oxidase system.[41,42] NADPH oxidase is one of the major sources of Ang II-mediated ROS in ventricular myocytes.[43] NADPH oxidase is a multicomponent enzyme complex that is composed of the membrane-bound heterodimer $gp91^{phox}$ (phox indicates phagocytic oxidase; Nox2) and its homologue Nox4; $p22^{phox}$; the cytosolic regulatory subunits $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$; and the small GTP-binding protein, Rac1.[44] Because NADPH oxidase is the main source of ROS in the cardiovascular tissues,[43] we next measured the protein expression of NADPH oxidase subunits $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$ in hearts by Western blotting. Our results showed that DNR increases the expression levels of NADPH oxidase subunits significantly and these effects are inhibited by combination with telmisartan. These data suggest that the increased level of NADPH subunits may contribute to increases in the level of ROS, which cause cardiac damage to DNR rats. The inhibition of Ang II with ARB may therefore be beneficial in reducing oxidative stress. However, the lack of evaluation of ROS production and NADPH oxidase activity can be considered as limitations of the present study.

Thioredoxin (Trx) regulates the DNA binding ability of certain transcription factors and counteracts oxidative stress by scavenging ROS and regulating enzymes that help to reduce oxidative stress.[45] Trx-R is the only enzyme in the cell known to reduce Trx. A recent study has demonstrated that Trx has pro-oxidant and pro-apoptotic properties in anthracycline chemotherapy.[46] In our study, DNR downregulated the expression of Trx-R and cotreatment with telmisartan reversed this effect. Moreover, to support our findings we examined myocardial 8-OHdG, which is a sensitive and specific marker of DNA damage induced by oxidative stress.[47] As we expected, the myocardial immunostaining for 8-OHdG expression was significantly increased in the DNR group. In contrast, myocardial 8-OHdG was decreased by the addition of telmisartan. Therefore, it is possible that the attenuation of myocardial 8-OHdG and increases in the levels of Trx-R protein expression by administration of telmisartan may be due to the antioxidant effect of this drug.

NF-κB is a rapidly inducible transcription factor that regulates the expression of various genes induced by genotoxic agents such as anthracycline drugs.[48,49] Ang II stimulation was also found to increase the translocation of the NF-κB p65 subunit to the nucleus.[50] In accordance with previous reports,[48–50] we observed activated NF-κB by increasing the protein expression of the NF-κB p65 subunit. Treatment with telmisartan inhibited this activation, which suggests that telmisartan can inhibit the activator signal transduction pathway of NF-κB. Further investigations are needed to confirm this hypothesis.

Recent studies have shown that anthracycline-induced cardiotoxicity decreases the expression of Bcl-2[51] and increases the expression of activated caspase-7, which induces apoptosis.[46] Several recent studies have shown that anthracyclines such as DOX and DNR induce apoptosis, which is considered to occur through the production of ROS.[10,24] Moreover, it is

believed that Ang II stimulation induces the apoptosis of ventricular myocytes in different animal models.[52–54] In the present study, we observed that telmisartan treatment significantly reduces DNR-induced myocyte apoptosis as revealed by the reduction in the number of apoptotic nuclei, as assessed by the TUNEL assay, a decreased level of activated caspase-7 and an increased level of Bcl-2 protein expression (Figure 1b, Figure 3 and Table 2). In addition, increased myocardial expression of AT-1R in DNR-treated rats was attenuated by cotreatment with telmisartan. These beneficial effects of telmisartan may be mediated by inhibition of the action of Ang II at AT-1R, a theory which is supported by previous animal experiments that demonstrate a key role for the local RAS in doxorubicin-induced cardiac injury.[13] As a consequence of inhibition of the action of Ang II, oxidative stress and myocardial apoptosis decreased.

Conclusions

The results of the present study demonstrate that telmisartan treatment may improve cardiac function by inhibiting the action of Ang II via AT-1R, which reverses oxidative stress and myocardial apoptosis, suggesting a beneficial effect of telmisartan treatment in the prevention of anthracycline-induced cardiotoxicity. We cannot rule out the beneficial effect of telmisartan in the prevention of DNR-induced cardiotoxicity being through its agonistic action on PPAR-γ activation, therefore the absence of an evaluation of the PPAR-γ activity can be considered a limitation of this study.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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