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Telmisartan prevents the progression of renal injury in daunorubicin rats with the alteration of angiotensin II and endothelin-1 receptor expression associated with its PPAR-γ agonist actions

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ABSTRACT

Angiotensin II (Ang II) receptor blocker (ARB) suppresses the progression of kidney disease. However, there is limited information regarding the nephroprotective effect of ARB in daunorubicin (DNR)-induced nephrotoxicity in rats. We examined the alteration of the renal Ang II and endothelin-1 (ET-1) receptor expression and the action of telmisartan, an ARB, on DNR-induced nephrotoxicity. Sprague–Dawley rats were treated with a cumulative dose of 9 mg/kg DNR (i.v.). Telmisartan was administered orally every day for 6 weeks. DNR rats showed nephrotoxicity as evidenced by worsening renal function, which was evaluated by measuring protein in urine, levels of urea and creatinine in serum, lipid profiles, malondialdehyde level, and the glutathione peroxidase activity in kidney tissue. These changes were reversed by treatment with telmisartan, which resulted in significant improvement in renal function. Furthermore, telmisartan increased nephrin protein expression, and down-regulated renal expression of Ang II and its receptor Ang II type I. Renal protein expressions of ET-1 and its receptor ET-receptor type A were increased in DNR rats, and treatment with telmisartan attenuated these increased expressions. Telmisartan mediated a further increase in the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ). In addition, the expressions of cyclooxygenase-2 and cellular adhesion molecules were increased in DNR rats, which were attenuated by telmisartan. In conclusion, telmisartan has a protective effect on DNR-induced nephrotoxicity through Ang II and ET-1, with the alteration of their receptor expressions, which is associated with its anti-inflammatory and anti-oxidant effects at least in part through PPAR-γ agonistic actions.

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1. Introduction

Daunorubicin (DNR), an anthracycline antibiotic, is an efficient drug for chemotherapy of acute lymphoblastic and myeloblastic leukemias (Laurent and Jaffezou, 2001). However, the use of cytostatic drug is limited by its free radical-induced toxicity, which also limits its dose and efficacy (Simunek et al., 2009). Furthermore, the optimal use of DNR is limited by a number of side effects; the most important are cardiotoxicity and nephrotoxicity, which eventually lead to congestive heart failure and renal failure, respectively (Arozal et al., 2010; Dziegiel et al., 2002a,b; Saad et al., 2006; Soga et al., 2005).

The exact mechanism of DNR-induced cardiotoxicity and nephrotoxicity has not been fully explored. However, several studies have suggested that formation of reactive oxygen species (ROS) through redox cycling of their aglycones as well as their anthracycline–iron complexes is the principal mechanism that induces cellular damage (Arozal et al., 2010; Dioudis et al., 1996; Dziegiel et al., 2002a,b; Ohtake et al., 1997; Saad et al., 2006; Simunek et al., 2009).

The efficacy of DNR as a cytotoxic agent against several types of human tumor has prompted intensive efforts in the search for drug treatments, which may reduce or prevent the development of cardiac and renal damage. Several strategies for reducing toxic-

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ity have been proposed, including dose-reduction protocol (Iarussi et al., 2005), encapsulating the drug with liposomes (Rahman et al., 2007; Siehl et al., 2005), and administration with anti-oxidant or metal chelators (Simunek et al., 2009). However, the protection afforded by these treatments has not been demonstrated to be consistently effective (Gianni et al., 2008).

The renin-angiotensin-aldosterone system is a major regulatory system of cardiovascular and renal functions. Its primary effector hormone, angiotensin II (Ang II), has several pathophysiological actions mediated by Ang II type I receptor (AT1R) and type 2 receptor (AT2R) in cardiovascular, atherosclerotic, and renal diseases (Atlas, 2007). In the kidney, activation of the Ang II–AT1R pathway results in hypertension, the production of pro-inflammatory mediators, cell proliferation, extracellular matrix synthesis, and release of ROS, which facilitate kidney damage and advance chronic kidney disease (Rüster and Wolf, 2006; Sachse and Wolf, 2007). A previous study reported that Ang II plays a key role in the process of anthracycline-induced cardiotoxicity (Toko et al., 2002). However, studies have suggested that angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) exert a protective role toward doxorubicin (DOX)-induced cardiotoxicity and nephrotoxicity (Ibrahim et al., 2009; Iqbal et al., 2008; Richard et al., 2008; Sacco et al., 2009), but these have not been investigated in DNR-induced nephrotoxicity. Furthermore, the exact mechanisms of action and efficacies of both ACEIs and ARBs in DNR-induced nephrotoxicity are not clear. Therefore, further experimental studies are needed in order to verify their efficacy and elucidate their exact mechanism of action.

Furthermore, ample evidence indicates that endothelin (ET) plays a role in the pathophysiology of various forms of renal disease, including chronic renal failure with hypertension (Goddard et al., 2004), renal fibrosis (Boffa et al., 2001), renal transplantation (Frank et al., 2006), nephritic syndrome (Vlachojannis et al., 1997), and cisplatin-induced nephrotoxicity (Lee and Ahn, 2008). The ET system includes ET isotypes (ET-1, ET-2, ET-3), ET converting enzyme, and its receptors (ET_AR, ET_BR). Stimulation of ET_A and ET_B elicits diverse physiological responses, including vasoconstriction, mitogenesis, inflammation, hypertrophy, and differentiation (Kirkby et al., 2008). In addition, ET-1 and Ang II are powerful vasoconstrictors involved in the regulation of vascular tone, and there is considerable evidence for an interaction between ET and renin-angiotensin system (RAS) (Suzuki et al., 2001).

Studies have shown that telmisartan is distinguished among other members of ARBs by its partial agonistic activity on peroxisome proliferator-activated receptor-gamma (PPAR-γ), which was reported to have anti-inflammatory and anti-oxidant properties (Cianchetti et al., 2008; Kobayashi et al., 2008; Yamagishi and Takeuchi, 2005). Partial agonism of PPAR-γ with telmisartan has been shown to reduce proteinuria, serum creatinine, and glomerulosclerosis in a non-diabetic model of glomerulosclerosis (Barnett, 2006) and inhibit oxidative stress and renal fibrosis in a model of unilateral ureteral obstruction (Sugiyama et al., 2005). All these findings suggest that telmisartan is more potent than other ARBs in protecting kidney structure and function in progressive proteinuric renal disease. However, to the best of our knowledge, there is only one published study that investigated the effect of telmisartan on DOX-induced nephrotoxicity (Ibrahim et al., 2009), but this has not been investigated in DNR-induced nephrotoxicity. Moreover, no studies have revealed the ARB action upon the alteration of renal Ang II, ET-1, and their receptor expression on the nephrotoxicity induced by anthracycline (DNR). Against this background, we plan to investigate the alteration of Ang II, ET-1, and their receptor expressions in kidney of DNR rats treated with vehicle or telmisartan, and to examine the efficacy of telmisartan against DNR-induced nephrotoxicity.

2. Materials and methods

2.1. 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 donated by Boehringer Ingelheim GmbH (Ingelheim am Rhein, Germany).

2.2. Animals and treatment

Eight-week-old male Sprague–Dawley rats were obtained from Charles River Japan Inc. (Kanagawa, Japan). The animals were quarantined and acclimatized for the additional 2 weeks prior to the initiation of the experiments. On day 0, each animal received a single intravenous injection of DNR at a dose of 3 mg/kg (i.v.). The drug was administered in three equal injections at 48 h intervals for a period of one week to achieve an accumulative dose of 9 mg/kg, which is well documented to produce cardiotoxicity and nephrotoxicity (Arozal et al., 2010; Soga et al., 2005). Age-matched rats were injected with corresponding volumes of 0.9% NaCl and used as a control (group Control; $n = 5$). Twenty-two DNR-treated rats were randomly divided into two groups and received oral administration of telmisartan (10 mg/kg/day; group DNR + Telm; $n = 10$) or vehicle (group DNR; $n = 12$). The dose of telmisartan was chosen on the basis of a previous report (Ibrahim et al., 2009). Administration of telmisartan was started on the same day as DNR administration and continued for 5 additional weeks after cessation of DNR administration (6 weeks total period). This duration of study was chosen on the basis of previous reports (Arozal et al., 2010; Giri et al., 2004; Nakhaoul et al., 2005). On day 41, rats were placed individually in metabolic cages for 24-h urine collections for the measurement of protein concentrations and body weight (BW) was measured. After the end of the study period (6 weeks), rats were sacrificed and kidney tissue was harvested for semi-quantitative immunoblotting and immunohistochemical studies. 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 committee of Niigata University of Pharmacy and Applied Life Sciences.

2.3. Estimation of biochemical parameters

Blood samples were collected in heparinized syringes by heart puncture. The collected blood was utilized for the subsequent determination of creatinine, total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-C), triglyceride, and blood urea nitrogen (BUN) and was stored at −80 °C. TC, LDL-C, and triglyceride levels were determined using standard enzymatic procedures. Urinary protein excretion was determined by the Bradford method (Bradford, 1976). Serum creatinine level was determined by the Jaffe method (Husdan and Rapoport, 1968). BUN was determined by the diacetyl monoxime method (Foster and Hochholzer, 1971).

2.4. Analysis of thiobarbituric acid reactive substances (TBARS)

The degree of lipid peroxidation was assessed using biochemical analysis of TBARS. TBARS are expressed in terms of malondialdehyde (MDA) level. The kidney tissue content of MDA was determined using TBARS assay kit (OXItex, ZeptoMetrix Corporation, New York, USA) in accordance with the manufacturer's instructions.

2.5. Measurement of total glutathione peroxidase (GPx) activity

Kidney tissue was homogenized in 6 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, USA). GPx activity in kidney tissue 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.

2.6. Histopathological analysis

The kidney tissue was decapsulated. Half of the kidney was immediately snap-frozen in liquid nitrogen for subsequent protein extraction and enzymatic assays. The remaining excised kidneys were cut into about 2-mm-thick transverse slices and fixed in 10% formalin. After being embedded in paraffin, several transverse sections were obtained from the kidney and stained with hematoxylin and eosin (H&E), and periodic acid Schiff (PAS) for histological evaluation, and also stained with Azan-Mallory to demonstrate fibrosis in kidney tissues. The frequency and the severity of lesions in kidney were assessed semi-quantitatively as previously reported (Arozal et al., 2010; Ibrahim et al., 2009) by light microscopy using the following scores: 0, normal; 1, mild; 2, moderate; and 3, severe. The criteria for kidney lesions were degree of glomerular congestion, tubular necrosis, size and frequency of protein casts in the cortical and medullary regions, intertubular hemorrhage, and degree of fibrosis.

2.7. Immunohistochemistry for Ang II, vascular cell adhesion molecule-1 (VCAM-1), and inter-cellular adhesion molecule-1 (ICAM-1)

Formalin-fixed, paraffin-embedded kidney tissue sections were used for immunohistochemical staining. After deparaffinization 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, rabbit polyclonal anti-Ang II antibody (Peninsula Laboratories Inc., San Carlos, CA, USA), anti-VCAM-1, or anti-ICAM-1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), diluted 1:50, at 4 °C, the slides were washed in TBS and then horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody was 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 hematoxylin. A negative control without primary antibody was included in the experiment to verify the antibody specificity.

2.8. Protein analysis by Western blotting

Protein lysate was prepared from kidney tissue as described previously (Gurusamy et al., 2004). The total protein concentration in samples was measured by the bicinchoninic acid method (Smith et al., 1985). For the determination of protein levels of AT1R, AT2R, ET-1, ET_AR, ET_BR, PPAR-γ, nephrin, cyclooxygenase-2 (COX-2), and transforming growth factor-beta 1 (TGF-β1), equal amounts of protein extracts (30 μg) were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (Bio-Rad, CA, USA) and transferred electrophoretically to nitrocellulose membranes. 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, USA) 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 HRP-conjugated secondary antibodies (Santa Cruz Biotechnology Inc.) and chemiluminescence developing agents (GE Healthcare, 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 Scion Image program (Epson GT-X700, Tokyo, Japan). Finally, Western blot data were normalized by those for renal GAPDH.

2.9. Statistical analysis

Data are presented as mean ± S.E.M., and were analyzed using one-way analysis of variance 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, USA) was used.

3. Results

3.1. Effect of telmisartan on BW and survival rate

Throughout the study period, BW was significantly decreased in DNR rats with or without telmisartan treatment compared with that of group Control rats. Although telmisartan treatment tended to increase BW compared with that in group DNR, the effect did not attain statistical significance (Table 1).

Six (50%) and none of 12 and 10 rats in groups DNR and DNR + Telm, respectively, died between days 14 and 42 (Table 1). None of the rats died in group Control. The mortality rate was higher in rats treated with DNR alone than that of group Control. The mortality rate was lower in group DNR + Telm than in group DNR rats.

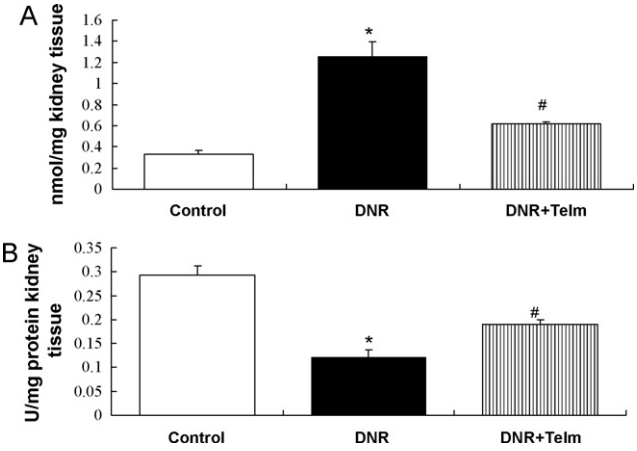

Fig. 1. Effects of telmisartan on lipid peroxidation as determined by measuring MDA levels (A) and GPx activities (B) in kidney homogenates from DNR-treated rats. The values are means ± S.E.M. group Control, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group DNR + Telm, DNR-treated rats administered with telmisartan (10 mg/kg/day). *$p < 0.05$ vs. group Control; #$p < 0.05$ vs. group DNR.

3.2. Effect of telmisartan on kidney function

Urinary protein excretion, serum creatinine, and BUN level were significantly increased in group DNR compared with those in group Control. In addition, LDL-C, TC, and triglyceride levels were also significantly increased in group DNR compared with those in group Control. Cotreatment of DNR-injected rats with telmisartan resulted in a significant decrease in urinary protein excretion, BUN, serum creatinine, LDL-C, and triglyceride levels compared with those of DNR-treated rats (Table 1).

3.3. Effects of telmisartan on renal levels of MDA and GPx activity

Administration of DNR caused a significant increase in MDA level in renal tissue compared with that in the control group. This was accompanied by a significant reduction in total GPx activity compared with that in the control group. Treatment with telmisartan significantly decreased MDA level and increased GPx activity compared with those in group DNR (Fig. 1A and B).

3.4. Effect of telmisartan against renal histological damage

The severity of kidney injury was investigated by examination of H&E, PAS, and Azan-Mallory staining. Renal tissues revealed

Table 1
Changes in survival rate, body weight and biochemical parameters after 6 weeks of treatment with telmisartan in DNR rats.

	Group Control $n = 5$	Group DNR $n = 12$	Group DNR + Telm $n = 10$
No. of rats died	0	6	0
Survival rate (%)	100	50	100
BW (g)	540 ± 8.9	366 ± 9*	385 ± 10
Protein urine excretion (mg/24 h)	37.5 ± 16.6	687.9 ± 51.5*	278.5 ± 25#
Serum creatinine (mg/dl)	1.1 ± 0.04	3.7 ± 0.7*	2.3 ± 0.3#
BUN (mg/dl)	23 ± 1.5	67 ± 3.4*	29 ± 2#
LDL-C (mg/dl)	7.6 ± 1.3	232.3 ± 20.6*	176 ± 18#
Total cholesterol (mg/dl)	53.3 ± 6.1	563.4 ± 33.7*	585 ± 59
Triglyceride (mg/dl)	46 ± 19.3	2690.8 ± 316*	947 ± 156#

Results are presented as the mean ± S.E.M. BUN, blood urea nitrogen; LDL-C, low density lipoprotein-cholesterol; group Control, aged matched normal rats; group DNR, DNR rats treated with vehicle; group DNR + Telm, DNR rats treated with telmisartan (10 mg/kg/day).

* $p < 0.05$ vs. group Control.

# $p < 0.05$ vs. group DNR.

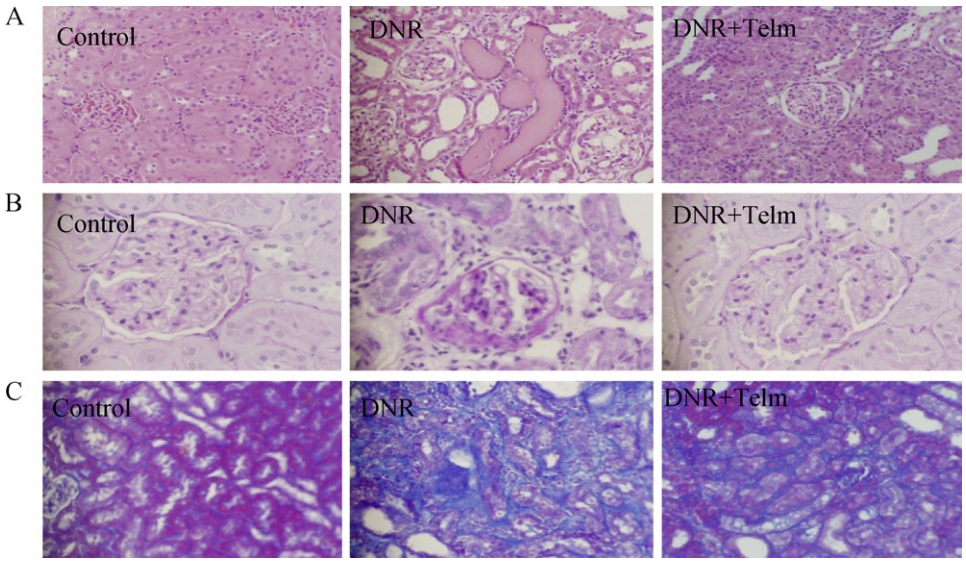

Fig. 2. (A) Hematoxylin and eosin staining of the cross-sectional tissue slices of kidney depicting glomerular congestion, tubular casts, and interstitial hemorrhage (200×). (B) Periodic acid Schiff staining of kidney depicting glomerular lesions (200×). (C) Azan-Mallory staining for fibrosis of the cross-sectional tissue slices of kidney. Fibrosis is indicated by the blue area as opposed to the red myocardium (200×). Group Control, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group DNR + Telm, DNR-treated rats administered with telmisartan (10 mg/kg/day).

normal histological findings in the control group. DNR administration resulted in substantial damage to kidney as indicated by glomerular congestion, tubular necrosis, large hyaline casts in tubular lumen, tubular dilatation, intertubular hemorrhage, and renal fibrosis (Table 2 and Fig. 2). Renal changes in group DNR were improved in the group treated with telmisartan (Table 2 and Fig. 2).

3.5. Effect of telmisartan on Ang II and its receptor

Renal immunoreactivity for Ang II was low or absent in the kidney of group Control. Kidney sections of group DNR showed stronger immunoreactivity for Ang II than those in group Control (Fig. 3A and D), and immunohistochemical analysis of the treatment group revealed a significant decrease in the renal level of Ang II (Fig. 3A and D). The renal expressions of AT1R and AT2R, as determined by Western blot, are shown in Fig. 4A–C. The AT1R expression was augmented transiently in the DNR group, and coadministration of telmisartan suppressed the enhancement of AT1R expression (Fig. 4A and B). Conversely, the expression of AT2R was not different among all groups (Fig. 4A and C).

3.6. Effects of telmisartan on endothelin system

Fig. 4A, D, E, and F shows the expressions of ET-1, ET_AR, and ET_BR in the kidney. The ET-1 protein expression was significantly increased in the kidneys of group DNR compared with that in group Control, which was counteracted by telmisartan treatment. The expression of ET_AR was significantly increased in kidneys of DNR rats, which was further decreased by cotreatment with telmisartan. Meanwhile, the expression of ET_BR was significantly increased in kidneys of DNR rats compared with that in group Control, which was further increased by cotreatment with telmisartan.

3.7. Effects of telmisartan on inflammatory markers

Renal immunoreactivies for VCAM-1 and ICAM-1 were increased in group DNR compared with those of group Control, which were attenuated by cotreatment with telmisartan (Fig. 3B and C). In addition, the renal expressions of COX-2 and TGF-β1 as assessed by Western blot were increased significantly in group DNR compared with those in group Control, which were significantly reduced further by cotreatment with telmisartan (Fig. 5A, D and E).

3.8. Effects of telmisartan on PPAR-γ and nephrin expression

Western blotting analysis showed that PPAR-γ protein expression was significantly increased in group DNR compared with that in group Control rats, which exhibited a further increase with the administration of telmisartan (Fig. 5A and B). Moreover, renal protein expression of nephrin was significantly decreased in group DNR compared with that in group Control rats. In contrast, treatment with telmisartan increased the protein level of nephrin compared with that in group DNR rats (Fig. 5A and C).

Table 2
Effect of telmisartan on histopathological changes in kidney tissues after 6 weeks of treatment in DNR rats.

Histopathological finding	Group Control $n = 5$	Group DNR $n = 6$	Group DNR + Telm $n = 10$
Glomerular congestion	0.0 ± 0.0	2.2 ± 0.2*	1 ± 0.3#
Tubular necrosis	0.0 ± 0.0	1.4 ± 0.24*	0.6 ± 0.2#
Hyaline casts	0.0 ± 0.0	2.8 ± 0.2*	1.6 ± 0.2#
Intertubular hemorrhage	0.0 ± 0.0	2.6 ± 0.24*	1.6 ± 0.4#
Renal fibrosis	0.0 ± 0.0	2.2 ± 0.2*	0.8 ± 0.2#

Results are presented as the mean ± S.E.M. group Control, aged matched normal rats; group DNR, DNR rats treated with vehicle; group DNR + Telm, DNR rats treated with telmisartan (10 mg/kg/day).

* $p < 0.05$ vs. group Control.

# $p < 0.05$ vs. group DNR.

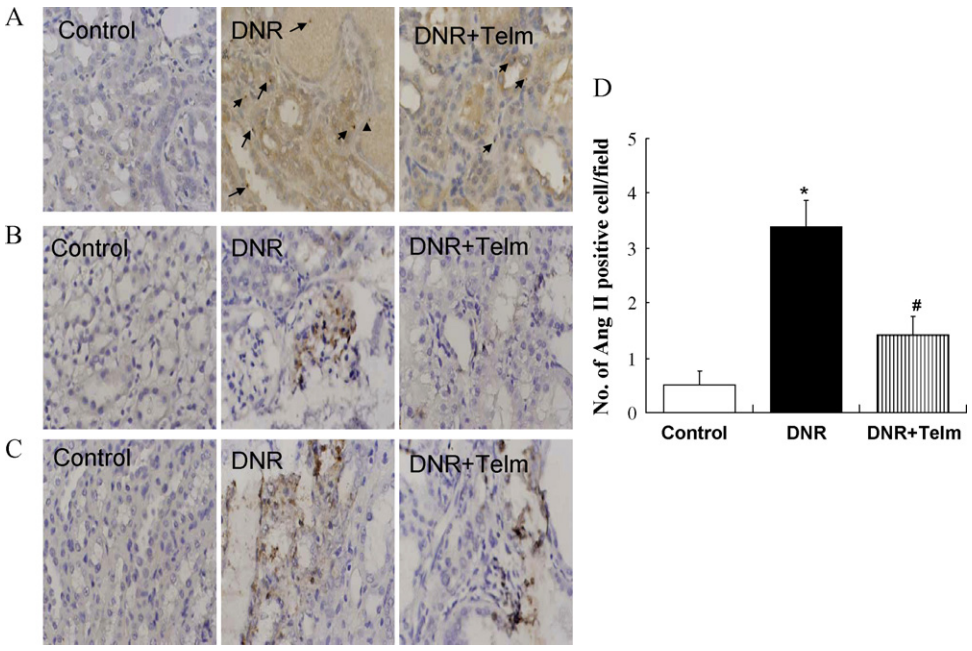

Fig. 3. (A) Renal levels of Ang II by immunohistochemical staining, and the positive immunostaining of Ang II exhibits a brown color (in color version) and is highlighted by arrow (400×). (B and C) Immunohistochemistry for VCAM-1 (B) and ICAM-1 (C) at 400× magnification. (D) Quantification of Ang II positive cell/field at 400× magnification. Group Control, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group DNR + Telm, DNR-treated rats administered with telmisartan (10 mg/kg/day). $^{*}p < 0.05$ vs. group C; $^{\#}p < 0.05$ vs. group DNR. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

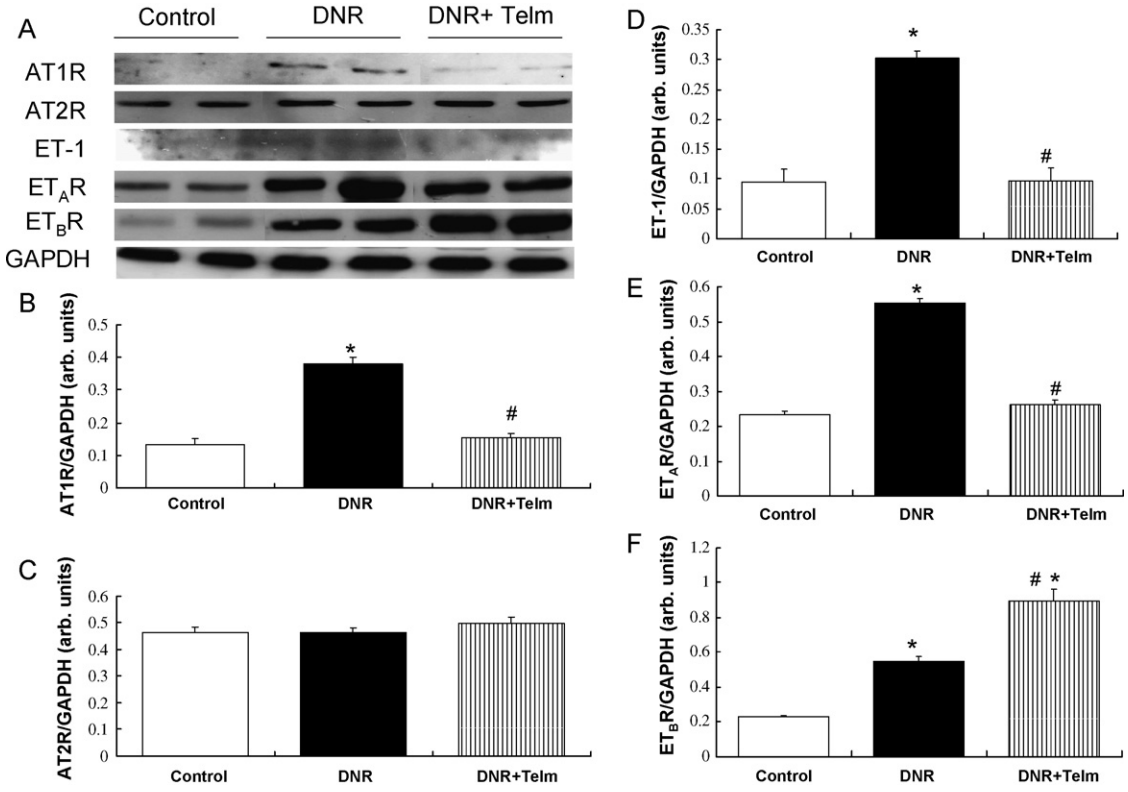

Fig. 4. Renal expressions of AT1R, AT2R, ET-1, ET_AR, and ET_BR. (A) Representative Western blots showing specific bands for AT1R, AT2R, ET-1, ET_AR, ET_BR and GAPDH as an internal control. Equal amounts of protein sample (30 μg) obtained from whole kidney homogenate were applied in each lane. These bands are representative of five separate experiments. (B–F) Densitometric data of protein analysis. The mean density values of AT1R, AT2R, ET-1, ET_AR, and ET_BR are expressed as ratios relative to that of GAPDH. Each bar represents mean ± S.E.M. group Control, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group DNR + Telm, DNR-treated rats administered with telmisartan (10 mg/kg/day). $^{*}p < 0.05$ vs. group Control; $^{\#}p < 0.05$ vs. group DNR.

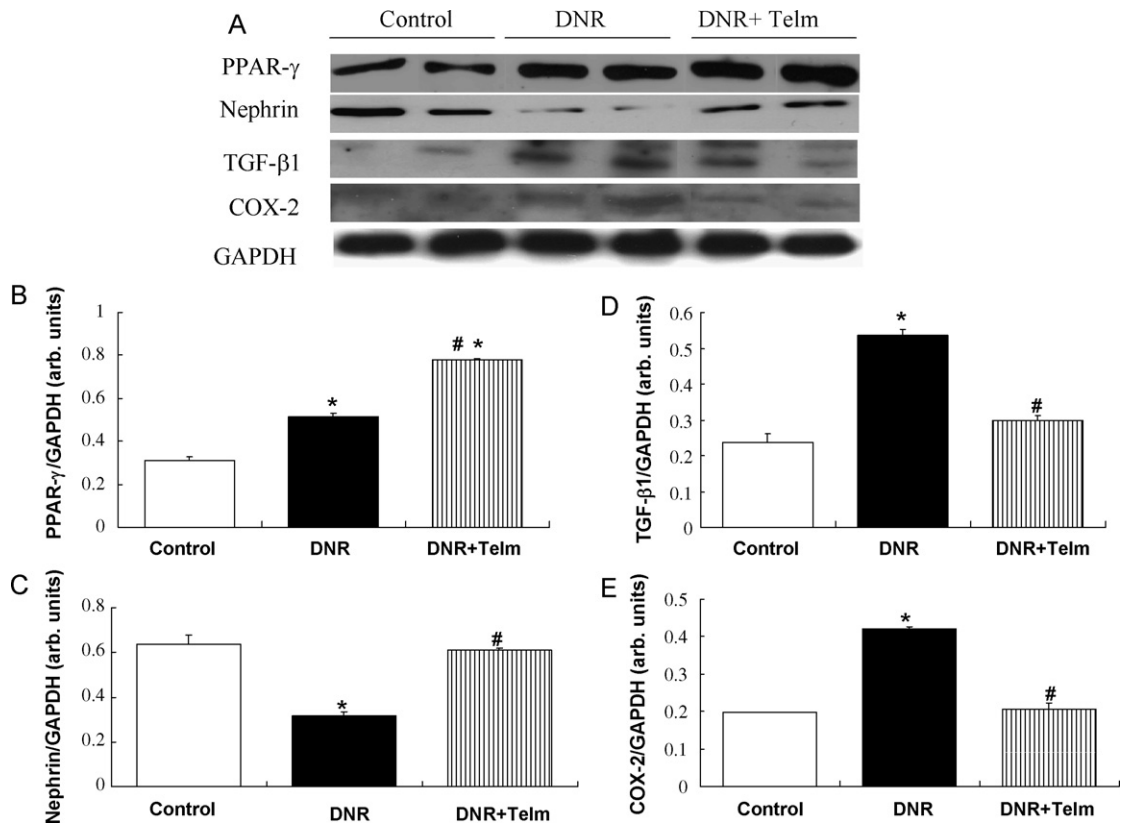

Fig. 5. Renal expressions of PPAR-γ, nephrin, TGF-β1 and COX-2. (A) Representative Western blots showing specific bands for PPAR-γ, nephrin, TGF-β1, COX-2 and GAPDH as an internal control. Equal amounts of protein sample obtained from whole kidney homogenate were applied in each lane. These bands are representative of five separate experiments. (B–F) Densitometric data of protein analysis. The mean density values of PPAR-γ, nephrin, TGF-β1 and COX-2 are expressed as ratios relative to that of GAPDH. Each bar represents mean ± S.E.M. group Control, age-matched normal rats; group DNR, DNR-treated rats administered with vehicle; group DNR + Telm, DNR-treated rats administered with telmisartan (10 mg/kg/day). $^{*}p < 0.05$ vs. group Control; $^{\#}p < 0.05$ vs. group DNR.

4. Discussion

This study provides in vivo evidence of the protective effects of telmisartan against DNR-induced nephrotoxicity. As expected, DNR administration led to impaired renal function and renal histological damage, as well as increased production of oxidative stress and pro-inflammatory cytokines. These changes were accompanied by the alteration of Ang II and ET-1 receptor expressions in kidney during administration of DNR with or without ARB treatment. Treatment with telmisartan showed protective effects against DNR-induced nephrotoxicity by reducing Ang II level and ET-1 expression through its receptors, and reducing inflammation and oxidative stress at least in part through its PPAR-γ agonistic action; as a consequence, the renal functions were improved and mortality rate was reduced.

Ang II is the major effector hormone of the RAS and contributes to a variety of renal and cardiovascular physiologic and pathologic mechanisms through stimulation of AT1 and AT2 receptors. The renoprotective effects of ARB have been shown using various clinical studies and experimental models of chronic kidney disease (Berl, 2009; Watanabe et al., 2009), including anthracycline-induced nephrotoxicity (Ibrahim et al., 2009; Mihailovic-Stanojevic et al., 2009). However, no detailed analysis of ARB action including the alteration of renal AT1R and AT2R expressions on the nephrotoxicity induced by anthracycline has been presented. In this study, we confirmed that the renal level of Ang II (Fig. 2A and D) and the renal expression of its receptor AT1 were increased in DNR rats, and cotreatment with telmisartan mediated the down-regulation of Ang II and AT1R without affecting the AT2R (Fig. 2A and 4A–C). AT1R is responsible for most of the pathophysiological actions of Ang II, and inappropriate activation of Ang II–AT1R pathways mediates the development and progression of chronic kidney disease (Kobori et al., 2007). Our results suggested that the AT1R expression in kidney is augmented in DNR rats, which may accelerate renal injury by Ang II; thus, telmisartan may exert renoprotective effects through inhibition of AT1R activation.

Ample evidence has shown that ET-1 expression is elevated in renal diseases such as ischemic acute renal failure (ARF), chronic transplant rejection, polycystic kidney disease, mesangial proliferative nephritis, aminonucleoside-induced nephrosis, and cisplastin-induced nephrotoxic ARF (Deng et al., 2000; Ebihara et al., 1997; Forbes et al., 2001; Lee and Ahn, 2008; Nakamura et al., 2010; Ong et al., 2003; Yoshimura et al., 1995); however, little is known about the expression of the ET system in anthracycline-induced cardiotoxicity (Bien et al., 2007), but not in nephrotoxicity. ET-1 directly inhibits Na-K-ATPase of renal tubular cells and modulates a variety of signal transduction pathways through its receptor as well (Garvin and Sanders, 1991). Numerous studies have shown that the up-regulation of the renal ET system exacerbates proteinuria (Goddard et al., 2004; Wenzel et al., 2009). Larivière et al. (1998) reported that rats with reduced renal mass show parallel reductions in proteinuria, vascular and glomerular prepro-ET-1 mRNA, and ET-1 peptide after RAS blockade with losartan and captopril. In addition, chronic ACE inhibitor treatment in animal models of glomerulosclerosis (Remuzzi et al., 1995) and immune-complex nephritis (Ruiz-Ortega et al., 1997) leads to a reduction in proteinuria as well as reductions in renal ET-1 mRNA and protein expression. In line with previous studies (Goddard et al., 2004; Larivière et al., 1998; Wenzel et al., 2009), we also found that renal expressions of ET-1 and ET_AR were up-regulated in DNR rats, which

were associated with increased urinary protein excretion (Table 1 and Fig. 4A, D–E) and treatment with telmisartan reduces the renal expressions of ET-1 and ET_AR and urinary protein excretion. These results indicate that the enhanced renal expression of ET-1 has a role in the development of renal injury, including proteinuria, in DNR rats and also provide evidence for an interaction between the ET and RAS in this animal model. Interestingly, the present study showed the increased expression of ET_BR by treatment with telmisartan. Previous study showed that the expression of ET_BR was increased in kidneys of deoxycorticosterone-salt rats, which were attenuated by rosiglitazone treatment (Bae et al., 2010). The reason behind why the increased expression of ET_BR in DNR rats treated with telmisartan remains to be determined, since we only measured the protein expression of ET_BR and did not measure the changes in gene level. As such, the net effect of telmisartan on ET_BR in our model is unclear, so further experiments will be required to clarify this issue.

Experimental investigation suggested that anthracycline-induced renal toxicities were associated with marked elevation in renal concentration of lipid peroxidation product (MDA) and a reduction in total GPx activity, which supported the hypothesis that oxidative stress and free radicals play a role in anthracycline toxicity (Arozal et al., 2010; Hou et al., 2009; Ibrahim et al., 2009; Mohan et al., 2010). Furthermore, Ang II is one of the most important known oxidative stress inducers Paravicini and Touyz (2006) and Toko et al. (2002) reported the non-toxic effect of DOX on cardiac muscle of AT1 knockout mice, indicating the participation of Ang II type 1a signaling pathway in the development of anthracycline-induced toxicity. Interestingly, we could observe elevated renal Ang II levels in the DNR rats which were associated with a marked increase in lipid peroxidation product MDA and a decrease in GPx activity (Figs. 1A and 3A and D), while coadministration of telmisartan with DNR significantly decreased renal Ang II levels, its receptor expression (AT1R), and MDA level and increased GPx activity. Decreased levels of lipid peroxidation product and increased GPx activity by telmisartan might contribute to a remarkable improvement of kidney function as shown by decreased urinary protein excretion and serum creatinine (Table 1). In addition, it has been reported that a reduction in glomerular nephrin expression is closely related to oxidative stress (Shibata et al., 2007). In our study, glomerular nephrin expression level was markedly reduced in group DNR with significant changes in renal histopathology. Telmisartan treatment significantly increased the protein expression level of nephrin. Moreover, telmisartan, as an ARB, has been proven to inhibit intracellular oxidative stress, at least in part, in a receptor-independent manner, possibly owing to its lipophilic and anti-oxidant structure (Shao et al., 2007). Thus, it is possible that the renoprotective effects of telmisartan also include induction of nephrin expression, which is considered to occur through its ability to reduce oxidative stress. Further studies are necessary to address this issue.

Anthracyclines such as DNR and DOX are also established tools to induce experimental nephrosis, which causes glomerular cell injury leading to gradual increase in proteinuria and subsequent/concomitant changes in plasma cholesterol, both of which have been shown in the present study. We speculate that the high levels of serum cholesterol in this study may be due to the increase in cholesterol synthesis by liver or increased absorption of cholesterol from intestine and the inhibition of conversion of cholesterol to bile acid in liver (Ito et al., 1991). Further studies are warranted to explore the exact mechanism behind this.

Telmisartan has been reported to have a partial agonistic effect on PPAR-γ (Benson et al., 2004). The ability of telmisartan to activate PPAR-γ seems to be independent of AT1R blocking action (Schupp et al., 2004). Furthermore, it has been demonstrated that telmisartan, suppressed the AT1R expression in both mRNA and protein levels through the PPAR-γ-mediated pathway and involvement of PPAR-γ in AT1R suppression has been further confirmed by using GW9662, a PPAR-γ antagonist (Imayama et al., 2006). The ability to activate PPAR-γ is reported to be unique to telmisartan among several AT1R antagonists (Benson et al., 2004). Activation of PPAR-γ exerts anti-inflammatory (Guyton et al., 2001), anti-fibrotic (Zheng et al., 2002), anti-oxidative and vasculo-protective effects (Tao et al., 2003) on different renal diseases, but little has been known about the effect of PPAR-γ agonist in anthracycline-induced nephrotoxicity. Activation of PPAR-γ was confirmed in the present study by Western blotting analysis and showed suppression of AT1R expression and increased PPAR-γ at the protein level after cotreatment with telmisartan in DNR rats. However, the reason behind the increased PPAR-γ expression in rats treated with DNR remains to be determined. In addition, because we only measured the expression of PPAR-γ at protein level and did not measure the activity of PPAR-γ, the net effect of overall PPAR-γ activity in our model is unclear. In light of this study limitation, further experiments will be required to understand whether the effects of telmisartan in our model are mediated by alterations in PPAR-γ activity.

Telmisartan has been reported to possess anti-inflammatory effects through its PPAR-γ activation (Cianchetti et al., 2008; Kobayashi et al., 2008; Tian et al., 2009; Yamagishi and Takeuchi, 2005). Furthermore, activation of PPAR-γ by both telmisartan and glitazones (rosiglitazone and pioglitazone) reduces inflammatory T-cell activation as well as chemokine-induced CD4-positive-lymphocyte migration and ICAM-3 translocation (Walcher et al., 2008). In vitro, Zou et al. (2010) reported that telmisartan and troglitazone have equal an anti-glomerulosclerosis effects in rat glomerular mesangial cells by blocking TGF-β and protein kinase A signaling. Similarly, the renoprotective effect of pioglitazone was demonstrated in a couple of experimental studies in which treatment with pioglitazone markedly reduced the occurrence of albuminuria and prevented the development of glomerulosclerosis and glomerular hypertrophy by suppressing several inflammatory and profibrotic genes including nuclear factor-κB, chemokine (C–C motif) ligand 2, TGF-β1, vascular endothelial growth factor, plasminogen activator inhibitor-1, type-IV collagen, and ICAM-1 in the kidney of diabetic rats with nephropathy (Ko et al., 2008; Ohga et al., 2007). In this study, coadministration of telmisartan with DNR inhibited inflammatory markers such as renal VCAM-1 and ICAM-1 levels and renal protein expression of COX-2 and TGF-β1, which suggest that it exerts anti-inflammatory effects through its PPAR-γ activation. Therefore, our study results suggest that the PPAR-γ agonist effect of telmisartan, provides an explanation for the anti-oxidative and anti-inflammatory effects of telmisartan in DNR rats.

In conclusion, we provide convincing evidence to suggest that telmisartan does indeed exert a protective effect against DNR-induced nephrotoxicity through Ang II and ET-1, with the alteration of their receptor expression, which is associated with its anti-inflammatory and anti-oxidant effects at least in part through PPAR-γ agonistic activity. On the basis of the present study, telmisartan may be used to prevent renal toxicity during administration of anthracycline as a chemotherapeutic agent for malignancies. In addition, it is of interest to evaluate the potential of other ARBs in preventing anthracycline-induced renal toxicity, since for more than a decade ARB have been known to have pronounced anti-proteinuric and renoprotective properties independently from their primary antihypertensive effect.

Conflict of interest

None.

Acknowledgements

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