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Comparative effects of torasemide and furosemide in rats with heart failure

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ABSTRACT

It has been reported that torasemide but not furosemide, may block the renin–angiotensin–aldosterone system and therefore it might attenuate myocardial remodeling accompanied by left ventricular (LV) dysfunction. We therefore compared the therapeutic effects of torasemide, a long-acting loop diuretic, and furosemide, a short-acting one, on the progression of LV remodeling in a rat model of chronic heart failure (CHF) after experimental autoimmune myocarditis (EAM). CHF was elicited in Lewis rats by immunization with porcine cardiac myosin. Twenty-eight days after immunization, rats were treated for 28 days with torasemide, furosemide, or vehicle. We investigated the effects on metabolic and neurohumoral parameters, cardiac fibrosis and remodeling in EAM rats. Diuresis was increased dose dependently by both torasemide and furosemide, showed an equipotent natriuretic effect. The urinary potassium excretion was significantly increased with furosemide in comparison to torasemide. Myocardial functional parameters were significantly improved by torasemide. Conversely, these parameters did not change in rats receiving furosemide. Torasemide suppressed LV fibrosis, myocardial protein levels of transforming growth factor-beta1, collagen III, and aldosterone synthase and improved survival rate to the control level, but furosemide did not. Moreover, both pharmacological interventions significantly elevated plasma angiotensin II and decreased atrial natriuretic peptide in a dose-dependent manner. Our results demonstrate that compared with furosemide, torasemide treatment significantly improved survival rate, LV function and ameliorated the progression of cardiac remodeling in rats with CHF after EAM.

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1. Introduction

Chronic heart failure (CHF) is a clinical syndrome caused by heart disease that is characterized by abnormal sodium and water retention resulting in edema [1]. In CHF patients, diminished cardiac output activates the sympathetic nervous system and renin–angiotensin–aldosterone system (RAAS) and the non-osmotic release of vasopressin. This causes a decrease in renal blood flow and an increase in the filtration rate resulting in an increase in water and sodium retention and consequently edema [2]. Diuretic therapy such as furosemide and torasemide is a core element in the treatment of CHF because diuretics relieve cardiac load by reducing water retention [3]. Torasemide exerts its action at the ascending limb of the loop of Henle, where it interacts with the Na^+, $2Cl^-$, K^+ co-transporter localized in the luminal surface [4]. It has a high bioavailability (>80%), and an elimination half-life of 3–4 h. Torasemide is reportedly more effective than furosemide in CHF with respect to reducing symptoms, hospitalization and other adverse cardiovascular events [5,6]. Recently, it has been reported that use of torasemide in the TORIC study was associated with lower mortality than furosemide [7], which suggests that torasemide has beneficial effects other than diuresis in patients with CHF. The difference in cardiac death between these two diuretics has been suggested to depend on the anti-aldosteronergic effect of torasemide. Furthermore, torasemide has been reported to attenuate left ventricular (LV) remodeling in patients with CHF to a greater extent than furosemide [8–10]. The effects of torasemide in CHF have been established in double-blind studies in comparison with furosemide, another loop diuretic of the same therapeutic class [5–9,11].

To date, the effects of torasemide and furosemide in a rat model of myosin-induced CHF are unknown. Cardiac myosin-induced experimental autoimmune myocarditis (EAM) is characterized by extensive myocardial necrosis, congestive heart failure and appearance of multinucleated giant cells and reflective to human giant cell myocarditis. Giant cell myocarditis is a fatal disorder, often leads to heart failure or arrhythmias [12]. EAM was demonstrated to progress into the clinicopathological state similar to dilated cardiomyopathy (DCM) in the chronic phase, and was characterized by the enlargement of the heart, dilatation of ventricles, diffuse and extensive myocardial fibrosis, and hypertrophic and atrophic changes of myocardial fibers, resembling human cardiomyopathy [13–15]. DCM is a serious disorder and the most common cause of heart failure. Difficulties in finding effective therapies are mainly based on the fact that the pathogenesis of DCM is still poorly understood. The establishment of an animal model that mimics human DCM will provide useful information with regard to the pathogenesis of DCM and the development of effective therapies. Thus the present study was designed to assess the comparative effects of torasemide, a long-acting loop diuretic, and furosemide, a short-acting one, on metabolic parameters and LV function using hemodynamic and echocardiographic parameters, neurohumoral factors such as plasma angiotensin II (Ang II), aldosterone and atrial natriuretic peptide (ANP) and protein levels of marker molecules of cardiac remodeling in a rat model of CHF after EAM.

2. Materials and methods

2.1. Materials

Unless otherwise stated all reagents were of analytical grade and were purchased from Sigma (Tokyo, Japan). Torasemide was provided by Taishotoyama Pharmaceutical Co. Ltd. (Toshima-Ku, Tokyo, Japan). Furosemide was purchased from Wako (Osaka, Japan).

2.2. Experimental design

All studies were carried out using 8-week-old male Lewis rats weighing about 230–250 g (Charles River Japan Inc., Kanagawa, Japan). CHF in rats was induced by immunization with porcine cardiac myosin into their footpads by subcutaneous injection. Porcine cardiac myosin was prepared from ventricular muscle according to the procedure described previously [13,14]. The morbidity of EAM was achieved 100% in rats immunized by this method. Rats immunized with myosin become ill and immobile on day 14, and their activity gradually recovered beginning at the fourth week. Twenty-eight days after immunization, the 59 surviving rats were randomly divided into five groups and received oral administration (p.o.) of torasemide (3 mg/(kg day), group T3, $n = 13$; 10 mg/(kg day), group T10, $n = 12$), furosemide (30 mg/(kg day), group F30, $n = 10$; 100 mg/(kg day), group F100, $n = 10$) or vehicle (group V, $n = 14$) for 28 days. Untreated age-matched Lewis rats were used as a normal control (group N, $n = 10$). The doses used in the experiments were determined on the basis of natriuretic and aldosterone binding properties demonstrated in an earlier report [16]. Throughout the study, all animals were cared for in accordance with the guidelines of our institute [14].

2.3. Diuretic study

The animals from groups V, T3, T10, F30 and F100 were placed individually in metabolic cages, and urine samples were collected for 5 and 24 h after drug administration on days 1 and 28. The urinary volume was noted and the sodium and potassium concentrations were measured using an electrolyte autoanalyzer (ATWill EA-06, Yokohama, Japan) from the samples collected during 5 h post dosing.

2.4. Hemodynamic and echocardiographic studies

Rats were anesthetized with 2% halothane in O_2 and subjected to surgical procedures to measure hemodynamic parameters on day 56. After the instrumentation, the concentration of halothane was reduced to 0.5% to record steady state hemodynamic data. Hemodynamic parameters such as mean blood pressure (MBP), peak LV pressure (LVP), central venous pressure (CVP), LV end-diastolic pressure (LVEDP) and the rate of intra-ventricular pressure rise and decline ($\pm dP/dt$) were recorded as previously described [14]. Two-dimensional echocardiographic studies were performed under 0.5% halothane 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, and 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) was calculated as diastolic dimension minus systolic dimension divided by diastolic dimension, expressed as a percentage. The study was performed in a blinded manner.

2.5. Histopathological analysis

After the measurement of hemodynamic and echocardiographic parameters, hearts were excised and weighed immediately (HW), and the heart weight to body weight ratio (H/B) was calculated. The excised hearts were cut into about 2-mm transverse slices and fixed in 10% formalin. After being embedded in paraffin, several transverse sections were obtained from the ventricle, and stained with Azan-Mallory staining. The area of myocardial fibrosis was measured quantitatively by a color image analyzer (CIA-102; Olympus, Tokyo, Japan), using the differences in color (blue fibrotic area opposed to red myocardium) of the photomicrographs of Azan-Mallory stained slides. The results are presented as the ratio of the fibrotic area to the whole area of the myocardium [14].

2.6. Estimation of neurohumoral parameters

Blood samples were collected in a heparinized syringe by heart puncture immediately after echocardiographic measurements. The collected blood was utilized for the purpose of subsequent determinations of plasma Ang II, aldosterone and ANP by standardized RIA [17] and EIA kit, respectively.

2.7. 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 Tris–HCl, 0.85% NaCl, pH 7.5) containing 0.1% bovine serum albumin (BSA). Endogenous peroxidase activity was quenched by incubating the slides in methanol and 0.6% H_2O_2 in methanol. To perform antigen retrieval, the sections were pretreated with trypsin for 15 min at 37 °C. After overnight incubation with the primary antibody, i.e., goat polyclonal anti-collagen III antibody (diluted 1:100) (Santa Cruz Biotechnology Inc., CA, USA) at 4 °C, the slides were washed in TBS and horseradish peroxidase (HRP)-conjugated rabbit antigoat 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 hematoxylin. A negative control without primary antibody was included in the experiment to verify the antibody specificity. Measurement of myocardial immunoreactivity for collagen III was performed in 100 randomly selected fields in heart sections in 400-fold magnification by light microscopy.

2.8. Western blotting

LV homogenates were prepared from rats treated as described above for 28 days and age-matched untreated normal control rats. For the determination of protein levels of aldosterone synthase (CYP11B2), transforming growth factor (TGF) β1, and collagen III, equal amounts of protein extracts (30 μg) were separated by 10%, 15% or 7.5% SDS-polyacrylamide gel electrophoresis (Bio-Rad, CA, USA), respectively, and electrophoretically transferred to nitrocellulose membranes. Membranes were blocked with 1% non-fat dry milk and 1% BSA (Sigma, Saint Louis, USA) in TBS-T (20 mM Tris, pH 7.6, 137 mM NaCl, and 0.05% Tween). All antibodies were purchased from Santa Cruz Biotechnology Inc., CA, USA aside from CYP11B2 (Chemicon International, CA, USA), and used at a dilution of 1:1000. After incubation with primary antibody, the bound antibody was visualized with the respective HRP-conjugated secondary antibodies (Santa Cruz Biotechnology Inc., CA, USA) and chemiluminescence developing agents (Amersham Biosciences, Buckinghamshire, UK). The level of GAPDH was estimated in every sample. 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 cardiac GAPDH.

2.9. Statistical analysis

All values are expressed as means ± S.E. Survival rate was analyzed by the Kaplan–Meier method. Statistical analysis of differences between the groups was performed by one-way ANOVA, followed by Tukey or Bonferroni methods of post hoc analysis and two-tailed *t*-test when appropriate. The Kruskal–Wallis test was used to analyze the survival times, as multiple groups were used. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Survival rate

The effects of torasemide and furosemide on survival rate are shown in Fig. 1 and Table 1. Five (35.7%), two (20%) and three (30%) of 14, 10 and 10 rats in groups V, F30 and F100, respectively, died between days 28 and 56 (Table 1). None of the rats died in groups T3, T10 or N (Table 1). Furosemide tended to improve the survival rate, but there was no statistically significant difference between vehicle and furosemide-treated CHF rats. In contrast, the survival rate improved significantly in the torasemide-treated rats than those in the vehicle and furosemide-treated rats (Fig. 1 and Table 1).

3.2. Diuretic action

The diuretic actions of torasemide and furosemide were compared in rats with CHF, and were shown in Fig. 2. In the CHF rats, both pharmacological interventions significantly increased urine volume and urinary sodium excretion in a dose-dependent manner during 5 h post dosing on days 1 and 28. On day 1, torasemide (3 mg/kg) significantly increased urine volume and urinary sodium excretion in comparison to furosemide (30 mg/kg). However, higher dose of both

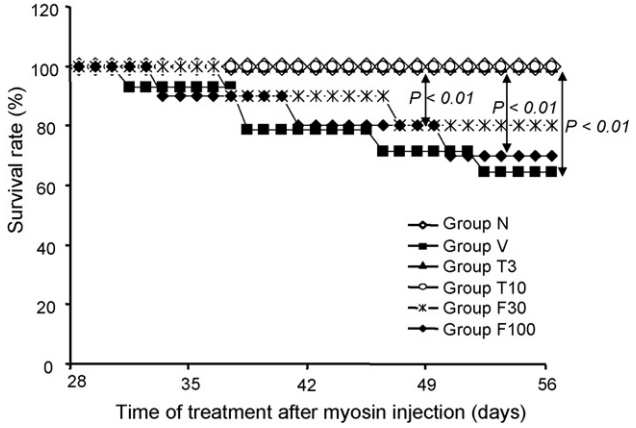

Fig. 1 – Effects of torasemide and furosemide on survival rate of rats with CHF induced by autoimmune myocarditis: five (35.7%), two (20%) and three (30%) of 14, 10 and 10 rats died in groups V, F30 and F100 from 28 to 56 days, respectively. No rats died in groups N, T3 and T10. The 56 days survival rates were significantly higher in groups N, T3 and T10 than in groups V, F30 and F100 ($P < 0.01$).

treatments equally increased urine volume and sodium excretion during 5 h post dosing on days 1 and 28 (Fig. 2A and B). On days 1 and 28, the 24 h urine volume with torasemide or furosemide was significantly increased (approximately 3–4.5- and 3-fold, respectively) in comparison with that in vehicle-treated CHF rats. Chronic treatment with torasemide, 3 and 10 mg/kg appeared equipotent to 30 and 100 mg/kg of furosemide, respectively, demonstrating that torasemide is at least 10 times more potent than furosemide in the CHF rats (Fig. 2A and B).

The urinary potassium excretion with torasemide or furosemide was significantly increased in a dose-dependent manner during 5 h post dosing on days 1 and 28. Chronic treatment with torasemide significantly decreased urinary potassium excretion in comparison to furosemide-treated rats (Fig. 2C).

3.3. *Effects of torasemide and furosemide on myocardial functions*

Although heart rate was not different among the six groups of rats, CVP and LVEDP were significantly higher and MBP, LVP and ±dP/dt were significantly lower in group V in comparison to group N indicating systolic and diastolic dysfunction in vehicle-treated rats (Table 1). CVP and LVEDP were significantly decreased in groups T3 and T10 compared to those in groups V, F30 and F100. However, MBP and LVP were not improved by the treatment. Torasemide improved ±dP/dt dose dependently, but the effect was significant only in group T10 in terms of −dP/dt. In contrast, hemodynamic parameters were not improved by furosemide-treatment in comparison to vehicle-treated rats (Table 1).

Echocardiographic data revealed that both LVDd ($P < 0.05$) and LVDs ($P < 0.01$) were increased in group V compared to group N. In addition, LV systolic function, as assessed by FS, was also reduced ($P < 0.01$) in group V compared to that in

Table 1 – Changes in survival rate, hemodynamic, echocardiographic and histopathological parameters after 4 weeks of treatment with torasemide and furosemide in rats with heart failure

	Group N ($n = 10$)	Group V ($n = 14$)	Group T3 ($n = 13$)	Group T10 ($n = 12$)	Group F30 ($n = 10$)	Group F100 ($n = 10$)
Number of dead animals per group	0	5	0	0	2	3
Survival rate (%)	100	64.3**	100$^{\#\#}$	100$^{\#\#}$	80$^{**\$\$\P\P}$	70$^{**\$\$\P\P}$
BW (g)	398 ± 7.4	312 ± 4.1**	278 ± 5.4$^{**\#\#}$	268 ± 3.8$^{**\#\#}$	300 ± 4.8$^{**\$\P\P}$	274 ± 7.8$^{**\#\#}$
HW (g)	1.011 ± 0.03	1.214 ± 0.04**	0.965 ± 0.03$^{\#\#}$	0.86 ± 0.03$^{\#\#}$	1.192 ± 0.05$^{**\$\$\P\P}$	0.977 ± 0.04$^{\#\#}$
H/B (g/kg)	2.485±0.06	3.904 ± 0.14**	3.486 ± 0.11$^{**\#}$	3.211 ± 0.07$^{**\#\#}$	3.798 ± 0.12**¶	3.498 ± 0.11$^{**\#}$
Area of fibrosis (%)	3.1±0.4	39 ± 2.5**	10 ± 1.2$^{*\#\#}$	5.7 ± 1.2$^{\#\#}$	28 ± 3.4$^{**\#\#\$\$\P\P}$	23 ± 4.3$^{**\#\#\$\$\P\P}$
CVP (mmHg)	0.09 ± 0.3	3.07 ± 0.6**	1.12 ± 0.4$^{\#}$	0.86 ± 0.3$^{\#}$	3.18 ± 0.4$^{**\$\$\P\P}$	3.54 ± 0.7$^{**\$\$\P\P}$
MBP (mmHg)	99 ± 2.5	81 ± 7.2*	87 ± 2.5	74 ± 5.8**	79 ± 3.5**	78 ± 2.6*
LVP (mmHg)	111 ± 2	95 ± 4*	97 ± 3.6	84 ± 6.6**	94 ± 4*	90 ± 3.4*
LVEDP (mmHg)	3.7 ± 0.8	11 ± 1.4**	5.7 ± 0.8$^{\#\#}$	4.3 ± 1.0$^{\#\#}$	10.8 ± 0.9$^{**\$\$\P\P}$	11.5 ± 0.7$^{**\$\$\P\P}$
+dP/dt (mmHg/s)	5984 ± 285	3816 ± 318**	5113 ± 350	5226 ± 86	4345 ± 352*	5557 ± 937
−dP/dt (mmHg/s)	5730 ± 253	3824 ± 360**	5535 ± 503	6171 ± 455$^{\#}$	4977 ± 448	4201 ± 746
HR (beats/min)	328 ± 12	341 ± 13	356 ± 9	316 ± 13	320 ± 18	340 ± 21
LVDd (mm)	6.8 ± 0.1	8.1 ± 0.2*	6.7 ± 0.3$^{\#}$	6.5 ± 0.4$^{\#\#}$	7.6 ± 0.4	7.7 ± 0.7
LVDs (mm)	4.1 ± 0.2	6.8 ± 0.3**	4.6 ± 0.2$^{\#\#}$	4.5 ± 0.5$^{\#\#}$	6.2 ± 5.9$^{**\$\$\P\P}$	6.1 ± 0.4$^{**\$\P\P}$
FS (%)	44 ± 1.8	16 ± 1.6**	32 ± 1.7$^{**\#\#}$	36 ± 0.9$^{*\#\#}$	20 ± 2.5$^{**\$\$\P\P}$	21 ± 2.5$^{**\$\$\P\P}$

Results are presented as the mean ± S.E. BW, body weight; HW, heart weight; HW/BW, ratio of heart weight to body weight; CVP, central venous pressure; MBP, mean blood pressure; LVP, left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; ±dP/dt, rate of intraventricular pressure rise and decline; HR, heart rate; LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; FS, fractional shortening; group N, aged matched untreated rats; group V, rats with heart failure treated with vehicle; groups T3 and T10, rats with heart failure treated with torasemide 3 and 10 mg/(kg day), respectively; groups F30 and F100, rats with heart failure treated with furosemide 30 and 100 mg/(kg day), respectively. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. normal; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. CHF; $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ vs. T3; $^{\P}P < 0.05$; $^{\P\P}P < 0.01$ vs. T10.

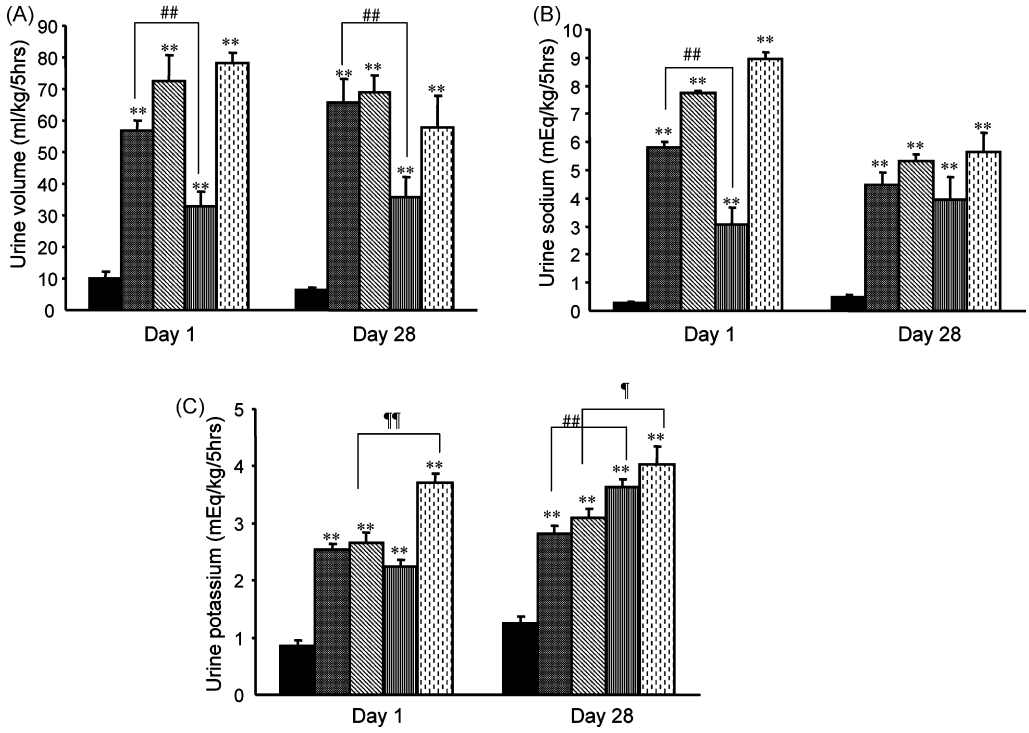

Fig. 2 – Diuretic actions (urine volume, A; urinary sodium, B; and urinary potassium, C) after p.o. administration of torasemide (n = 6/group), furosemide (n = 6/group) and vehicle (n = 6) for 5 h in rats with CHF. The values are means ± S.E. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle-treated CHF rats (■); $^{\#\#}P < 0.01$ vs. group T3 (▩), rats treated with torasemide (3 mg/(kg day)); $^{¶}P < 0.05$ and $^{¶¶}P < 0.01$ vs. group T10 (▨), rats treated with torasemide (10 mg/(kg day)); (▥), rats treated with furosemide (30 mg/(kg day)); (□), rats treated with furosemide (100 mg/(kg day)).

group N (Table 1). Torasemide treatment significantly reduced both LVDd and LVDs and increased FS compared to those in groups V, F30 and F100. Although, furosemide treatment reduced those parameters (LVDd and LVDs) and increased FS compared to those in group V, the effects attained were not statistically significant (Table 1).

3.4. *Assessment of neurohumoral parameters*

The plasma aldosterone concentrations in group V did not differ from those in group N; however Ang II and ANP concentrations were significantly elevated in group V in comparison to group N (Fig. 3A–C). Both pharmacological interventions significantly elevated plasma concentration of Ang II and decreased ANP concentration in comparison to those in group V (Fig. 3A and C). On the contrary, plasma concentration of aldosterone was significantly increased only in the group treated with torasemide in comparison with that in group V (Fig. 3B).

3.5. *Histopathology*

HW and H/B were significantly larger in group V than in group N rats (Table 1). Both treatments reduced HW and H/B in a dose-dependent manner, and the effect was significant in groups T3, T10 and F100 when compared with group V. HW was significantly lower in groups T3 and T10 than in groups V and F30, in which it was comparable to that in group N rats (Table 1).

The hearts from group V rats showed massive fibrosis compared to those from group N rats (Fig. 4A). The percent area of fibrosis was significantly lower in the furosemide-treated rats than in vehicle-treated rats, but the effect was still significantly higher than those in group N rats. The percent area of fibrosis was significantly lower in groups T3 and T10 than in groups V, F30 and F100, in which it was comparable to that in group N rats. Among the five groups treated with vehicle or diuretics, the area of fibrosis was lowest in group T10 (Figs. 4A and 5A and Table 1).

3.6. *Immunohistochemistry*

Myocardial immunoreactivity for collagen III was little or absent in the hearts of group N. Midventricle sections of group V showed stronger immunoreactivity for collagen III than those of group N (Fig. 4B). Immunohistochemical analysis of the torasemide groups revealed a significant and dose-dependent decrease in the myocardial level of collagen III (Figs. 4B and 5B) and the effect was up to the control level. Myocardial immunoreactivity for collagen III was also significantly decreased in the furosemide-treated rats than in the vehicle-treated rats, but this effect was still significantly higher than those in group N rats (Figs. 4B and 5B).

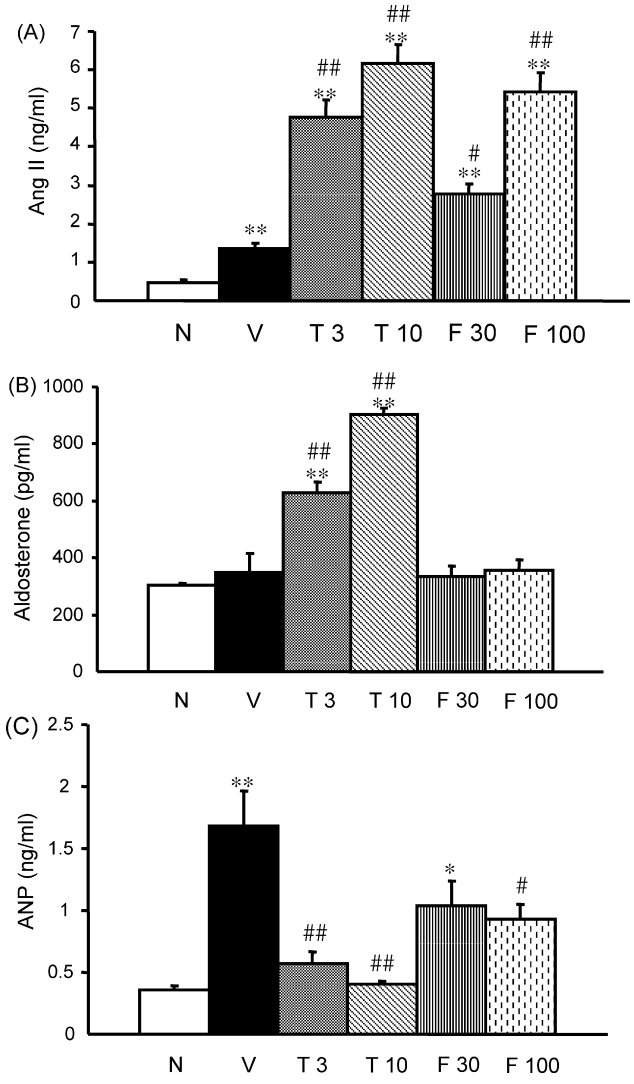

Fig. 3 – Effects of torasemide and furosemide on neurohumoral parameters: plasma Ang II (A), aldosterone (B) and ANP (C) concentration. Group N, age-matched untreated rats; group V, myosin-injected rats administered with vehicle; groups T3 and T10, rats with CHF treated with torasemide (3 and 10 mg/(kg day), respectively); groups F30 and F100, rats with CHF treated with furosemide (30 and 100 mg/(kg day), respectively). The values are means ± S.E. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. group N; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. group V.

3.7. *TGF β1, collagen III, and CYP11B2 protein levels assessed by Western blotting*

Western blotting analysis showed that TGF β1, collagen III, and CYP11B2 protein levels were significantly increased in vehicle-treated rats compared to group N rats (Fig. 6). Treatment with torasemide significantly decreased the myocardial levels of TGF β1, collagen III and CYP11B2 protein, and the effects were reached up to the control level. Myocardial levels of TGF β1, collagen III were also significantly decreased in the furosemide-treated rats than in the vehicle-treated rats, but this effect was still significantly higher than those in group N rats. However, myocardial levels of CYP11B2 were not decreased in groups F30 and F100 from those in group V rats (Fig. 6).

4. Discussion

The results of our study demonstrated that both torasemide and furosemide caused a potent diuretic effect, however the improvement of survival, myocardial function and attenuation of cardiac remodeling were seen only with torasemide treatment in rats with CHF after EAM. LV systolic and diastolic dysfunction is common in patients with DCM and is related to cardiac symptoms and prognosis [18]. Torasemide, not furosemide, improved both systolic (+dP/dt, LVDs and %FS) and diastolic (−dP/dt, LVEDP and LVDd) function of myocardium and these changes were associated with improvement in the rate of survival (Table 1). Thus, the present data suggest that torasemide offers additional benefits regarding mortality, morbidity and functional improvement over furosemide. Moreover, improvement of −dP/dt with torasemide treatment was negatively correlated with collagen III ($r = -0.78$, $P < 0.065$; $r = -0.84$, $P < 0.037$) measured by western blotting and immunohistochemistry, respectively. These results suggest that torasemide may have direct effects on myocardial relaxation. Murray et al. and others reported that torasemide is more effective than furosemide in CHF with respect to reducing symptoms, hospitalization and other adverse cardiovascular events [5,6]. Several recent experimental studies reported lack of benefits and presence of adverse events for furosemide, a short-acting loop diuretic, in heart failure models [19,20]. Thus, we hypothesized that the use of short-acting loop diuretics might explain the poor outcomes of patients treated with non-potassium-sparing diuretics described in the previously described retrospective studies [21,22]. Thus, current results are partly compatible with clinical studies that torasemide, a long-acting loop diuretic, is favorable for the treatment of CHF compared with furosemide.

Torasemide is a novel diuretic which has a potent and long-lasting diuretic action [23], which is achieved by inhibiting the reabsorption of water and electrolytes in the distal tubules, including loop of Henle [24]. Ghys et al. [25] have shown that i.v. injection of torasemide produces less kaliuresis than does furosemide at doses that cause an equivalent level of natriuresis and diuresis in anesthetized rats. In the present study, the potassium loss with torasemide is lower than with furosemide, despite similar aquaresis and natriuresis at the dosages used (Fig. 2). Moreover, it has been reported that torasemide causes significant, dose-dependent inhibition of the amount of receptor-bound aldosterone, whereas furosemide has no effect of aldosterone receptors [16]. This may explain the decreased urinary potassium excretion with torasemide. Our data indicate that torasemide has a diuretic effect equivalent to 1/10 of the dose of furosemide. Along with enhanced diuresis, there was a relevant reduction in body weight (BW) (Table 1), increased natriuresis and improvement in the rate of survival confirming the effectiveness of torasemide in rats with CHF.

RAAS affects myocardial fibrosis and plays an important role in both diastolic and systolic dysfunction [26] and has

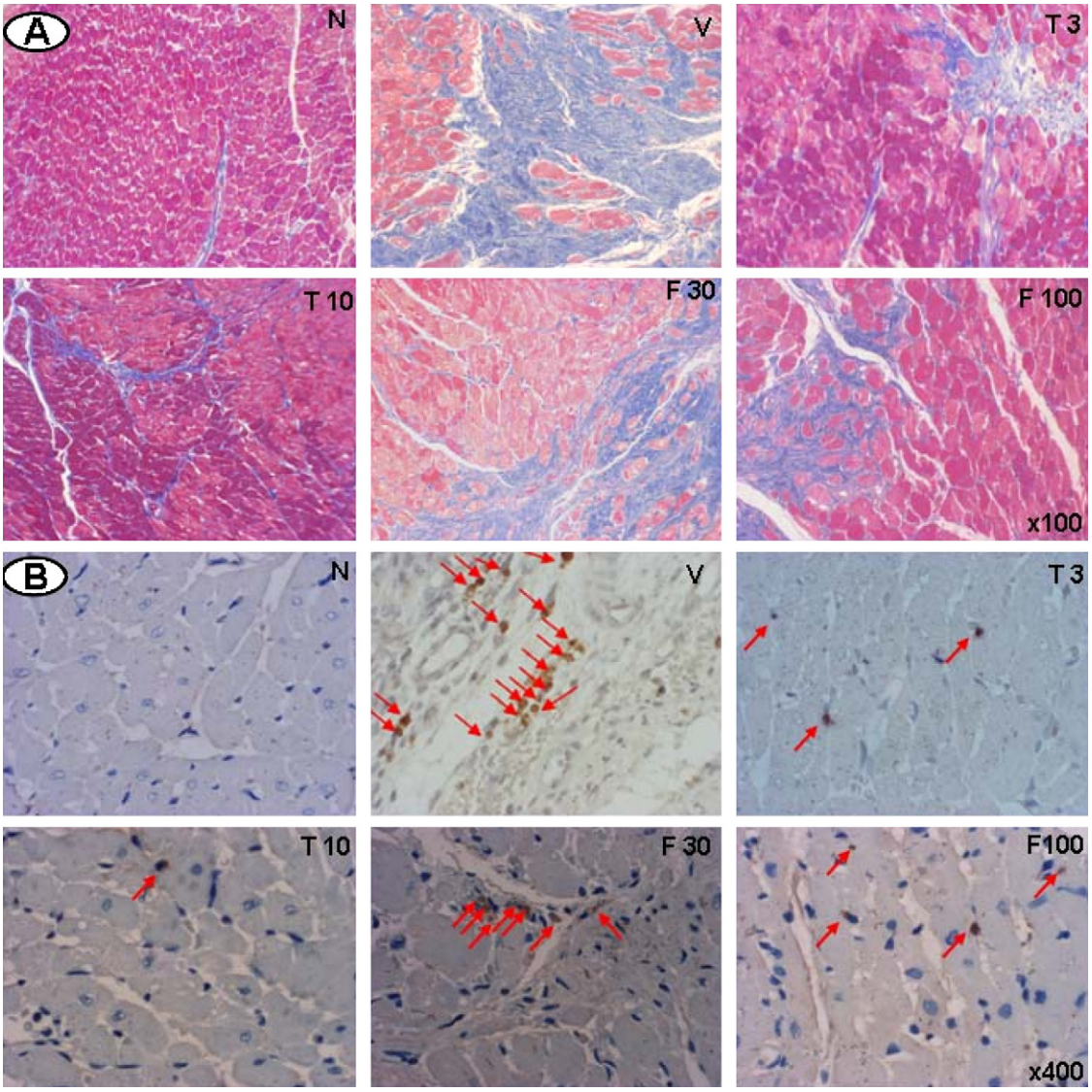

Fig. 4 – (A) Azan-Mallory staining for fibrosis of the cross-sectional tissue slices of hearts. Fibrosis is indicated by blue area as opposed to the red myocardium (100×). (B) Myocardial levels of collagen III by immunohistochemical staining, and the positive immunostaining of collagen III exhibit brown color and highlighted by red arrows (400×). Group N, age-matched untreated rats; group V, myosin-injected rats administered with vehicle; groups T3 and T10, rats with CHF treated with torasemide (3 and 10 mg/(kg day), respectively); groups F30 and F100, rats with CHF treated with furosemide (30 and 100 mg/(kg day), respectively).

adverse clinical consequences that result in increases in deaths caused by progressive heart failure. Activation of RAAS promotes structural remodeling of the heart and the progression of heart failure. It has been proposed that increase in myocardial fibrosis during heart failure is due to both increased collagen synthesis by fibroblasts and unchanged or decreased fibrillar collagen degradation [27]. For instance, a numerous findings suggest that aldosterone may play an important role in myocardial fibrosis, which leads to LV remodeling and results in LV dysfunction [26,28,29]. Recently, it has been reported that aldosterone is produced in the ventricle of the failing human [30] and rat hearts [31]. Additionally, CYP11B2 is detected in the hearts of postmyocarditis rats [32]. Satoh et al. [33] reported that cardiac CYP11B2 expression positively correlates with the degree of myocardial fibrosis which results in LV dysfunction in patients with CHF.

Yamato et al. and others reported that torasemide attenuates LV remodeling in patients with CHF in comparison with furosemide treatment [8,9,11]. Moreover, very interestingly, Lopez et al. reported that torasemide's abilities differ from furosemide's in reversing myocardial fibrosis and reducing collagen synthesis in patients with CHF [9]. Myocardial fibrosis, the hallmark of DCM, is observed in dilated cardiomyopathic hearts as indicated by Azan-Mallory staining and increased myocardial TGF β1 and collagen III levels (Figs. 4–6). Torasemide attenuated LV fibrosis and its marker molecules (TGF β1 and collagen III) to the control level and improved myocardial function. Recently, it has been reported

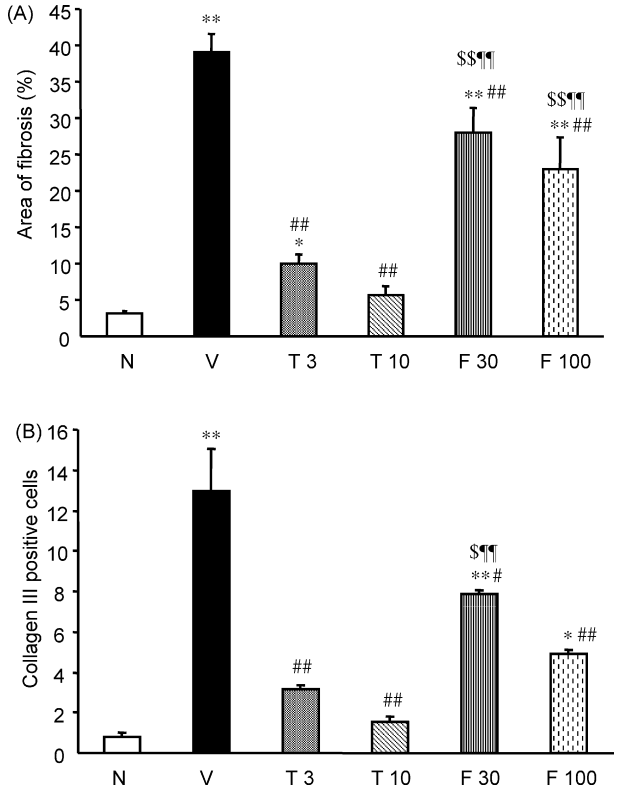

Fig. 5 – Effects of torasemide and furosemide on myocardial remodeling (fibrosis) and its marker protein (collagen III) levels in rats with CHF induced by autoimmune myocarditis. Bar graph shows quantitative analysis of fibrosis (A), and collagen III (B). Each bar represents means ± S.E. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. group N; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. group V. $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ vs. group T3; $^{\P\P}P < 0.01$ vs. group T10. Group N, age-matched untreated rats; group V, myosin-injected rats administered with vehicle; groups T3 and T10, CHF rats treated with torasemide (3 and 10 mg/(kg day), respectively); groups F30 and F100, CHF rats treated with furosemide (30 and 100 mg/(kg day), respectively).

that azosemide, another long-acting loop diuretic like torasemide attenuated LV fibrosis to a greater extent in comparison to furosemide in rats with heart failure [34]. Although, in our study, furosemide decreased LV fibrosis and its marker molecules than in the vehicle-treated rats, but the reductions were still significantly higher than those in the control rats (Figs. 4–6 and Table 1). The results of Fig. 4 are in agreement with the previous studies in which the cardiac collagen metabolism is deeply modified by torasemide [9]. We could observe a positive correlation between cardiac CYP11B2 expression, severity of LV dysfunction and the degree of myocardial fibrosis ($r = 0.990$; $P < 0.001$) in rats with CHF. These results are in consistent with the previous study [33]; in addition CYP11B2 expression and cardiac fibrosis are found to be decreased more with torasemide than those of furosemide treatment. Consequently, torasemide might inhibit the production and secretion of aldosterone locally, which could inhibit myocardial fibrosis. Our findings demonstrate that torasemide exerts an antifibrotic effect which results in improvement of survival rate. This is supported by changes in parameters assessing cardiac function (Table 1).

Aldosterone directly affects cardiac tissues and causes the development of cardiac hypertrophy, fibrosis and heart failure [35]. Importantly, the severity of LV remodeling seems to be closely associated with cardiac CYP11B2 and aldosterone rather than plasma levels, which did not differ between sham-operated and rats with myocardial infarction [36]. The post infarction rise in cardiac aldosterone can be prevented by an Ang II type 1 receptor antagonist, suggesting that the increase in tissue Ang II may be directly responsible [36]. Our results showed that aldosterone synthase (CYP11B2) was significantly higher in the hearts of group V than those in group N rats, although plasma aldosterone was not significantly different between groups N and V. Treatment with torasemide significantly reduced myocardial expression of CYP11B2. Taken together, these previous data and our present results suggest that locally produced aldosterone in CHF rats could play a primary role in the pathogenesis of LV remodeling in an autocrine or paracrine manner. Furthermore, rats receiving a 7-day course of torasemide had higher plasma aldosterone concentrations [37], and similar findings have been reported in humans [8], but furosemide lacks these properties [8,37]. In the present study, torasemide treatment significantly increased plasma aldosterone concentrations along with a significant reduction in CYP11B2 protein levels (Figs. 3 and 6), whereas furosemide does not. We assumed that the increase in Ang II after treatment with furosemide and torasemide may be as compensation to decreased circulatory blood volume. Moreover, we cannot exclude the possibility that reduction in plasma sodium might have stimulated renin release which in turn increased in plasma Ang II (data not shown). The increase in aldosterone concentration with torasemide treatment is may be due to prevention of binding of circulatory aldosterone to its receptor [38], may reflect results of a potential decrease in endogenous aldosterone [8], however furosemide did not alter plasma aldosterone level. Very recently, Kasama et al. reported that compared with furosemide, torasemide treatment can ameliorate cardiac sympathetic activity and LV remodeling in patients with CHF [11]. The current results may suggest that sympathetic activation preceded deleterious pathways in association with the furosemide treatment, a short-acting loop diuretic, resulting in poor outcomes on survival. Interestingly, it has been shown that transcardiac extraction of aldosterone is reduced in CHF patients treated with torasemide [10] and that torasemide blocks the binding of the hormone to its mineralocorticoid receptor [8,16,39]. Even though, torasemide is a potent loop diuretic, it causes less hypokalemia than furosemide, which is consistent with the contention that torasemide blocks aldosterone receptors. On the other hand, it has been reported that in vitro, torasemide, but not furosemide, inhibits Ang II-induced vasoconstriction and intracellular Ca^{2+} increase in the aorta of spontaneously hypertensive rats [40]. Therefore, the possibility exists that the ability of torasemide to decrease myocardial fibrosis may also be related to interference with activity of humoral profibrotic factors including Ang II [41], as well as aldosterone [28,29].

Plasma ANP concentration is a useful prognostic indicator in patients with CHF, and is documented to be elevated in

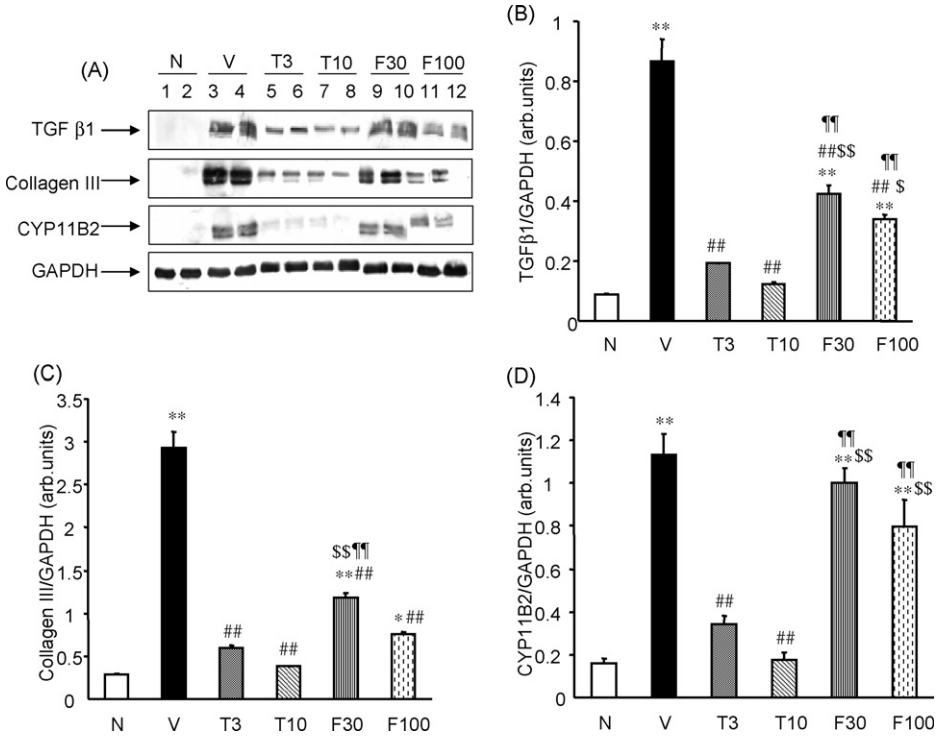

Fig. 6 – . Myocardial expressions of TGF β1, collagen III, and CYP11B2 proteins. (A), Representative Western blots showing specific bands for TGF β1, collagen III, and CYP11B2, and GAPDH as an internal control. An equal amount of protein sample obtained from left ventricular homogenate was applied in each lane. Group N (Lanes 1 and 2), age-matched untreated rats; group V (Lanes 3 and 4), myosin-injected rats administered with vehicle; group T3 (Lanes 5 and 6), rats treated with torasemide (3 mg/(kg day)); group T10 (Lanes 7 and 8), rats treated with torasemide (10 mg/(kg day)); group F30 (Lanes 9 and 10), rats treated with furosemide (30 mg/(kg day)); group F100 (Lanes 11 and 12), rats treated with furosemide (100 mg/(kg day)), respectively. These bands are representative of five separate experiments. (B–D) Densitometric data of protein analysis. The mean density value of TGF β1, collagen III, and CYP11B2 was expressed as a relative ratio to that of GAPDH. Each bar represents means ± S.E. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. group N; $^{\#\#}P < 0.01$ vs. group V; $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ vs. group T3; $^{\P\P}P < 0.01$ vs. group T10.

cardiac hypertrophy or failure [42]. Vehicle-treated rats had developed cardiac hypertrophy and LV dilation, evidenced by an increase in ANP levels, HW, H/B, LVDd, and LVDs, and decrease in FS (Fig. 3C and Table 1). In comparison with furosemide, torasemide-treated rats showed a significant reduction in those parameters and increase in the FS have been observed. In the high-dose furosemide group, those parameters (LVDd, LVDs and FS) were not different from vehicle-treated rats but ANP levels, HW and H/B were significantly decreased. These results indicated that torasemide improves myocardial systolic and diastolic function, and attenuates abnormal cardiac hypertrophy.

In conclusion, our results indicate that compared with furosemide, torasemide treatment significantly improved survival rate, LV function and ameliorated the progression of cardiac remodeling (fibrosis and hypertrophy) beyond its renal effects in rats with CHF after EAM.

Acknowledgments

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