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Full Paper

Tolvaptan Attenuates Left Ventricular Fibrosis After Acute Myocardial Infarction in Rats

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Abstract. Tolvaptan, a non-peptide V_2-receptor antagonist, is a newly developed diuretic agent. Recently, we reported that tolvaptan has diuretic as well as anti-inflammatory and anti-fibrotic actions in chronic heart failure. In this study, we investigated whether tolvaptan has a cardioprotective effect in acute heart failure after myocardial infarction (MI). After MI induction, rats were randomized into 6 groups as follows: vehicle group, group treated with 15 $mg \cdot kg^{-1} \cdot day^{-1}$ furosemide, 2 groups treated with 3 or 10 $mg \cdot kg^{-1} \cdot day^{-1}$ tolvaptan, and 2 groups treated with 15 $mg \cdot kg^{-1} \cdot day^{-1}$ furosemide combined with 3 or 10 $mg \cdot kg^{-1} \cdot day^{-1}$ tolvaptan. Each agent was administered for 2 weeks, and blood pressure levels and infarct sizes were similar in all MI groups. Lower left ventricular end-systolic volumes and greater improvement of left ventricular ejection fraction were observed in the tolvaptan-treated groups compared with the vehicle group. In contrast, furosemide alone did not improve them. Sirius red staining revealed that tolvaptan significantly repressed MI-induced interstitial fibrosis in the left ventricle. MI-induced mRNA expressions related to cardiac load, inflammation, and fibrosis were significantly attenuated in the combination group. The combination treatment also repressed MI-induced mineralocorticoid receptor expression. Tolvaptan, or combination of furosemide and tolvaptan, may improve cardiac function in acute MI.

Keywords: arginine vasopressin, acute myocardial infarction, diuretic, tolvaptan, cardiac remodeling

Introduction

Acute myocardial infarction (MI) frequently progresses to congestive heart failure (HF). Acute congestive HF may aggravate respiratory status and left ventricular (LV) function and reduce survival (1 – 2). To improve pulmonary congestion, loop diuretics are often used. However, furosemide, which is the most commonly used loop diuretic, induces natriuresis, which results in decreased serum sodium levels and activation of the renin–angiotensin–aldosterone system (RAAS), suggesting that furosemide may accelerate cardiac remodeling by RAAS activation (3). Furthermore, furosemide increases the risk of renal dysfunction, and impaired renal function is a stronger predictor of mortality than impaired cardiac function (4).

Arginine vasopressin (AVP) is known to play an important role in water metabolism by inducing water reabsorption at the renal collecting duct via the V_2 receptor (V_2R). AVP is also involved in the maintenance of blood pressure (BP) via the V_{1a} receptor ($V_{1a}R$) in the vasculature (5 – 6). Several studies have shown

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significantly higher plasma AVP levels in patients with chronic HF as well as acute HF, which may lead to volume overload (7 – 9). A non-peptide AVP V_2R antagonist, tolvaptan (6, 10), does not affect the RAAS. The large, multi-centre trial Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) revealed that chronic tolvaptan administration was safe and induced aquaresis, which was evidenced by body weight reduction during the initial hospitalization period following acute decompensated HF. Additionally, tolvaptan improved dyspnoea, although it neither improved nor worsened the primary outcome variable, mortality (11 – 13).

We have reported that tolvaptan may have anti-inflammatory and anti-fibrotic actions in chronic LV dysfunction after MI in rats (14). It could attenuate infiltration of macrophages by suppressing monocyte chemoattractant protein-1 (MCP-1) expression and interstitial fibrosis by suppressing transforming growth factor-β1 (TGF-β1) expression in the marginal infarct region. Furthermore, these actions might be associated with suppression of $V_{1a}R$ and endothelin-1 (ET-1) mRNA expression (14). However, whether tolvaptan has a cardioprotective effect on acute HF remains unknown.

In this study, we investigated whether tolvaptan could attenuate MI-induced fibrosis, inflammation, and LV dysfunction in the acute phase. We obtained evidence of improvement of acute HF after MI, suggesting a beneficial effect of tolvaptan.

Materials and Methods

Animals and experimental design

Furosemide was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and tolvaptan was prepared by Otsuka Pharmaceutical Co., Ltd. (Tokushima). All procedures were in accordance with the Osaka City University animal care guidelines, which conform to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Male Wistar rats, aged 8 – 9 weeks and weighing 260 – 290 g, were purchased from CLEA Japan, Inc. (Tokyo). After administering pentobarbital sodium [50 $mg \cdot kg^{-1}$, intraperitoneally (i.p.)] as an anaesthetic, MI was induced by ligation of the left coronary artery as described previously (14 – 15).

On the day after MI induction, MI-affected rats were randomized into 6 groups: a non-treated vehicle (V) group, a group treated with 15 $mg \cdot kg^{-1} \cdot day^{-1}$ furosemide (F group), 2 groups treated with 3 or 10 $mg \cdot kg^{-1} \cdot day^{-1}$ tolvaptan (T3 or T10 groups, respectively), and 2 groups treated with a combination of 15 $mg \cdot kg^{-1} \cdot day^{-1}$ furosemide and 3 or 10 $mg \cdot kg^{-1} \cdot day^{-1}$ tolvaptan (FT3 or FT10 groups, respectively). We used 3 or 10 $mg \cdot kg^{-1} \cdot day^{-1}$ tolvaptan in the present study because these doses significantly increased urine volume in rats; 1 $mg \cdot kg^{-1} \cdot day^{-1}$ tolvaptan did not alter urine output (16). Furthermore, the diuretic action of 3 $mg \cdot kg^{-1}$ tolvaptan appeared similar to that of 15 $mg \cdot kg^{-1}$ furosemide. Excluding the suturing of the coronary artery, the same surgical procedure was performed on a control (C) group of age-matched rats. The bait, prepared by Oriental Yeast Co., Ltd. (Tokyo), was mixed with the respective treatment agents and administered to the MI-affected rats. The rats were individually placed in metabolic cages and had free access to food and water, such that assessment of the fluid and food intake and urine volume was possible.

Two weeks after treatment, blood pressure (BP) and heart rate (HR) were measured. Cardiac function was monitored by echocardiography as described below. Immediately following echocardiography, the rat abdomen was cut open and a blood sample was collected from the inferior vena cava. The heart was then immediately excised, and the ventricle was separated from the atrium and weighed. Infarct size was calculated as the ratio of the scar area to the entire cardiac muscle area that was measured as described previously (14 – 15). The ventricle was separated into the upper and lower portions; then, the upper portion of the left ventricle was divided into the marginal zone and non-infarcted zone. The specimens obtained were immediately frozen in liquid nitrogen and stored at −80°C until use. The lower portion was fixed in 10% formaldehyde overnight and embedded in paraffin.

Echocardiographic study

Transthoracic echocardiographic studies were performed on rats according to a previously described method (14 – 15). Briefly, rats were anesthetized with tiletamine (10 $mg \cdot kg^{-1}$, i.p.) and xylazine (10 $mg \cdot kg^{-1}$, i.p.). A two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscles. LV ejection fraction (LVEF) was calculated by measuring the LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV) by using a modified Simpson's method.

Blood measurements

At 2 weeks after each treatment, the collected serum or plasma was used for measurement of blood urea nitrogen (BUN), creatinine, sodium (Na), potassium (K), chlorine (Cl), osmolality, brain natriuretic peptide (BNP), plasma renin activity (PRA), plasma aldosterone concentration (PAC) (Enzo Life Sciences Inc., Plymouth

Table 1. Metabolic parameters and body weight

	C (n = 7)	MI					
		V (n = 6)	F (n = 6)	T3 (n = 7)	T10 (n = 7)	FT3 (n = 8)	FT10 (n = 6)
Drinking volume (mL·day^{-1})	30.2 ± 0.7	28.3 ± 2.0	28.4 ± 2.7	28.7 ± 1.5	32 ± 1.4	31.9 ± 2.2	37.9 ± 2.2*
Urine volume (mL·day^{-1})	12.7 ± 0.9	10.2 ± 0.2	10.4 ± 0.1	11.5 ± 0.4	13.9 ± 1.6	13.8 ± 0.9	17.2 ± 1.4*
Food intake (g·day^{-1})	25.4 ± 0.6*	19.1 ± 0.3	20.1 ± 0.4	20.2 ± 0.4	20.4 ± 0.5	19.7 ± 0.7	20 ± 0.5
Body weight (g)	331.7 ± 4.2*	310.7 ± 4.9	307.5 ± 3.2	323 ± 4.2	321.3 ± 7.3	309.9 ± 3.8	317.5 ± 4.5

Values are mean ± S.E.M. *$P < 0.05$ vs. V group. C, sham-operated control rats; V, non-treated vehicle rats with MI; F, 15 mg·kg^{-1}·day^{-1} furosemide–treated rats with MI; T3 or T10, 3 or 10 mg·kg^{-1}·day^{-1} tolvaptan–treated rats with MI, respectively; FT3 or FT10, a combination of 15 mg·kg^{-1}·day^{-1} furosemide and 3 or 10 mg·kg^{-1}·day^{-1} tolvaptan–treated rats with MI, respectively. *$P < 0.05$ vs. V group.

Meeting, PA, USA), and copeptin (Peninsula Laboratories, San Carlos, CA, USA).

Estimation of cardiac fibrosis

The area of interstitial fibrosis in the marginal area of the infarct was measured as described previously (14, 17). Briefly, 4-μm-thick sections were cut and stained with Sirius red stain for the measurement of the area of interstitial fibrosis. The area of interstitial fibrosis was calculated as the ratio of the sum of the total area of interstitial fibrosis to the sum of the total connective tissue area plus the area of cardiomyocytes in the marginal area of the left ventricle. Each field was analyzed using image-analysing software (Micro Analyzer; Japan Poladigital, Tokyo).

RNA preparation and analysis

RNA from the marginal area of the LV was isolated using ISOGEN (Nippon Gene, Toyama) (14 – 15, 18). To determine gene expression levels, we subjected the RNA samples to quantitative real-time RT-PCR (qRT-PCR, 7500 Fast; Applied Biosystems, Carlsbad, CA, USA). One-step qRT-PCR reactions were performed using 100 ng of total RNA per reaction. TaqMan primers and probes used in this study were previously described (14). For normalization, the transcript levels were compared to those of GAPDH.

Statistical analyses

All data are presented as means ± S.E.M. Intergroup comparisons were made using one-way analysis of variance followed by Fisher's protected least significant difference test by using StatView (SAS Institute, Inc., Cary, NC, USA). Differences were considered statistically significant at $P < 0.05$.

Results

Effects of tolvaptan and furosemide on metabolic parameters

Measurements of the metabolic parameters 2 weeks after treatment are shown in Table 1. Because the food intake in all MI groups was similar, the planned treatment was successfully administered to MI rats. Body weight (BW) was also similar in all MI groups. Compared to the V group, the FT10 group showed significantly increased drinking and urine volumes.

Effects of tolvaptan and furosemide on hemodynamic and organ weights

Compared to the V group, the treated groups showed no significant differences in the HR and systolic BP (Fig. 1). Furthermore, compared to the V group, the F, T3, T10, FT3, and FT10 groups showed similar infarct sizes (39.2% ± 1.5%, 35.0% ± 3.4%, 35.4% ± 2.8%, 34.4% ± 2.2%, 36.9% ± 2.5%, and 37.2% ± 3.3%, respectively) (Fig. 1D). Thus, the MI-induced increase in the value of ventricular weight·BW^{-1} was inhibited by high-dose tolvaptan and combination therapy.

Neither lung weights nor liver weights were significantly different between the MI groups (data not shown).

Blood chemical analysis

Measurements of the blood and urine chemical parameters are shown in Table 2. Intergroup differences in BUN and serum creatinine levels were not significant. Furthermore, intergroup differences in serum electrolyte parameters were not significant. The level of BNP, which is a marker for cardiac load, in the V group was higher than that in the C group, while those in the T3 and T10 groups were lower than that in the V group. PRA level was significantly higher in the combination therapy groups than in the V group. There was no significant difference in the PAC between the C and V groups, whereas the PAC was significantly elevated in the F

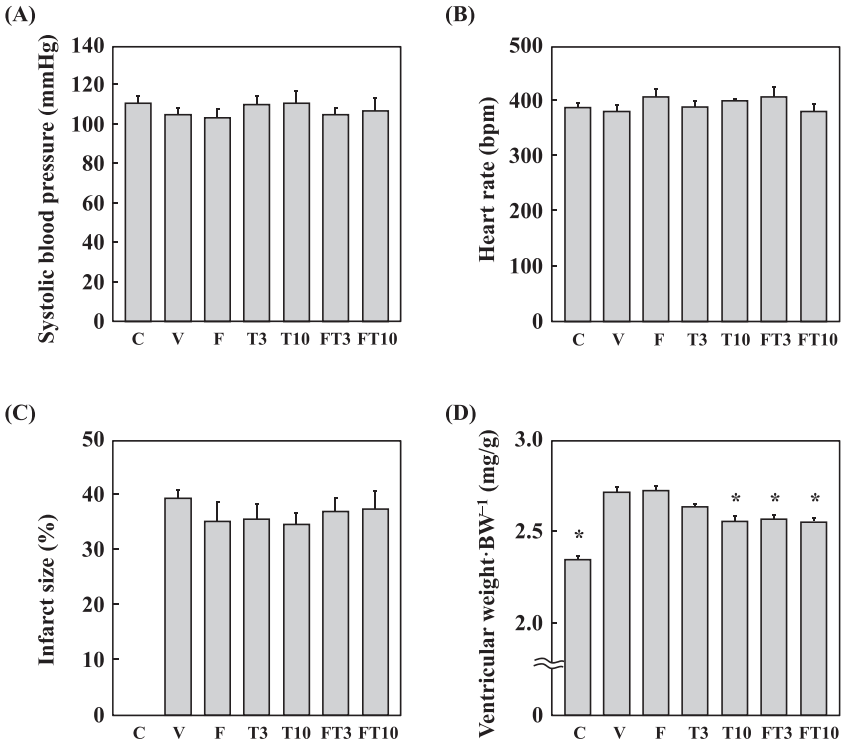

Fig. 1. Hemodynamics, infarct size, and ventricular weights. Systolic blood pressure (A), heart rate (B), and myocardial infarct sizes (C) were measured. No significant intergroup differences were noted. D) The value of ventricular weight / body weight (BW) in the V group was significantly higher than that in the C group. This increase was significantly suppressed in the T10, FT3, and FT10 groups. C, sham-operated control rats; V, non-treated vehicle rats with MI; F, 15 mg·kg^{-1}·day^{-1} furosemide–treated rats with MI; T3 or T10, 3 or 10 mg·kg^{-1}·day^{-1} tolvaptan–treated rats with MI, respectively; FT3 or FT10, a combination of 15 mg·kg^{-1}·day^{-1} furosemide and 3 or 10 mg·kg^{-1}·day^{-1} tolvaptan–treated rats with MI, respectively. Values are the mean ± S.E.M. (n = 6 – 8). *P < 0.05 vs. V group.

Table 2. Blood chemical parameters

	C (n = 7)	MI					
		V (n = 6)	F (n = 6)	T3 (n = 7)	T10 (n = 7)	FT3 (n = 8)	FT10 (n = 6)
Serum							
BUN (mg·dL^{-1})	20.1 ± 0.7	17.3 ± 0.6	20.7 ± 0.8	19.4 ± 0.8	18.6 ± 0.7	21.1 ± 0.8	20.7 ± 0.7
Cre (mg·dL^{-1})	0.22 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.24 ± 0.01
Na (mEq·L^{-1})	141.0 ± 0.5	141.2 ± 0.7	140.8 ± 0.6	140.4 ± 0.9	140.7 ± 0.6	141.9 ± 0.5	142.2 ± 0.6
K (mEq·L^{-1})	5.3 ± 0.3	5.0 ± 0.2	5.9 ± 0.3	7.0 ± 0.3	6.8 ± 0.4	5.4 ± 0.1	5.3 ± 0.2
Cl (mEq·L^{-1})	103.1 ± 0.5	103.2 ± 0.3	102.2 ± 0.3	104.1 ± 1.1	103.6 ± 0.6	103.0 ± 0.5	103.2 ± 0.4
Osm (mOsmkg·H_2O^{-1})	303.9 ± 1.1	300.7 ± 1.2	301.0 ± 0.7	304.9 ± 1.0*	303.6 ± 1.3	304.8 ± 1.0*	302.2 ± 0.9
Plasma							
BNP (pg·L^{-1})	115.7 ± 3.7*	155.0 ± 8.5	143.3 ± 7.1	131.4 ± 4.6*	131.4 ± 8.3*	140.0 ± 7.6	148.3 ± 9.8
PRA (ng·mL·h^{-1})	2.1 ± 0.5	2.2 ± 0.4	4.0 ± 0.7	2.8 ± 0.4	3.6 ± 0.5	4.1 ± 0.5*	4.4 ± 1.0*
Aldosterone (pg·mL^{-1})	179.0 ± 23.1	207.8 ± 19.0	350.3 ± 51.5*	368.7 ± 25.7*	315.6 ± 59.4*	203.9 ± 22.3	183.3 ± 15.2
Copeptin (pg·mL^{-1})	1.0 ± 0.1*	3.7 ± 1.1	1.0 ± 0.1*	4.1 ± 1.8	3.4 ± 0.6	2.0 ± 0.7	0.9 ± 0.1*

Values are the mean ± S.E.M. MI, myocardial infarction; C, sham-operated control rats; V, non-treated vehicle rats with MI; F, 15 mg·kg^{-1}·day^{-1} furosemide–treated rats with MI; T3 or T10, 3 or 10 mg·kg^{-1}·day^{-1} tolvaptan–treated rats with MI, respectively; FT3 or FT10, a combination of 15 mg·kg^{-1}·day^{-1} furosemide and 3 or 10 mg·kg^{-1}·day^{-1} tolvaptan–treated rats with MI, respectively. *P < 0.05 vs. V group.

and T3 groups compared with that in the V group. Plasma level of copeptin, the C-terminal part of the AVP precursor peptide, was significantly increased by MI. Furosemide significantly reduced the copeptin level, but tolvaptan alone did not change it.

Cardiac function

Figure 2 shows the LVEDV, LVESV, and LVEF measurements obtained by echocardiographic studies. Although the LVEDV did not show any significant differences compared to the V group, the T10 and FT10 groups had significantly lower LVESV (Fig. 2B). More-

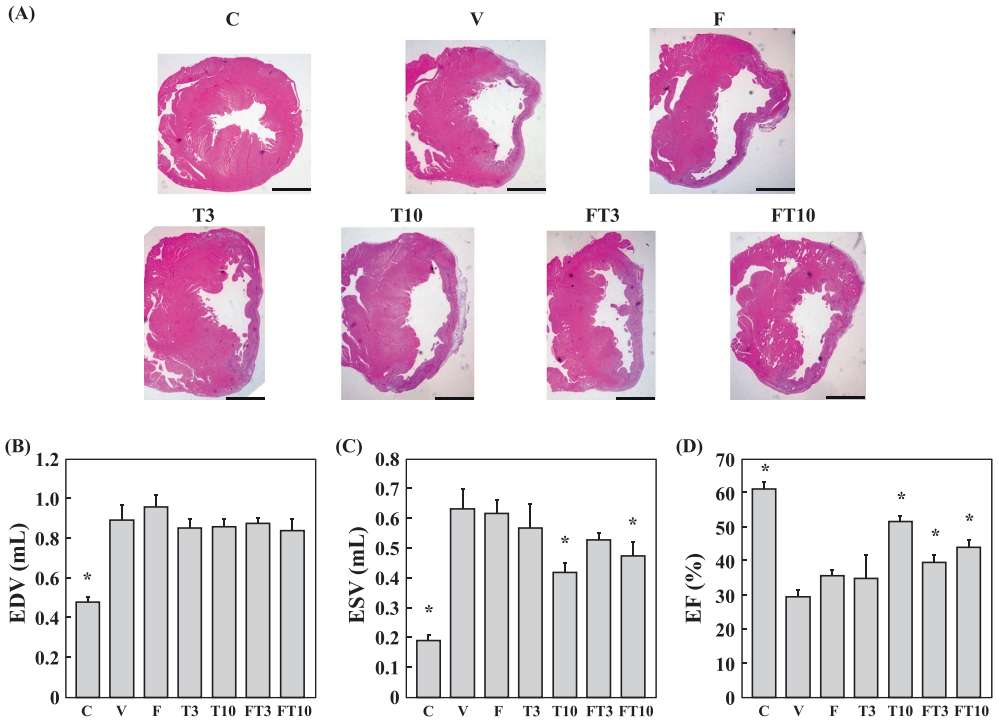

Fig. 2. Echocardiographic measurements at 2 weeks after treatment. A) Representative photomicrographs of the cross-sections (original magnification × 12.5) with hematoxylin–eosin staining. Bar, 1 mm. Graphs show echocardiographic assessments of LVEDV (A), LVESV (B), and LVEF (C) in rats. LVEDV and LVESV were significantly higher in group V than group C. LVESV was significantly lower in the T10 and FT10 groups than in the V group. C) LVEF was significantly decreased in the V group compared to that of the C group. T10, FT3, and FT10 groups had significantly greater improvement in LVEF than the V group. Values are reported as the mean ± S.E.M. LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction. Other abbreviations are the same as in the Fig. 1 legend. Values are reported as the mean ± S.E.M. (n = 6 – 8). **P* < 0.05 vs. V.

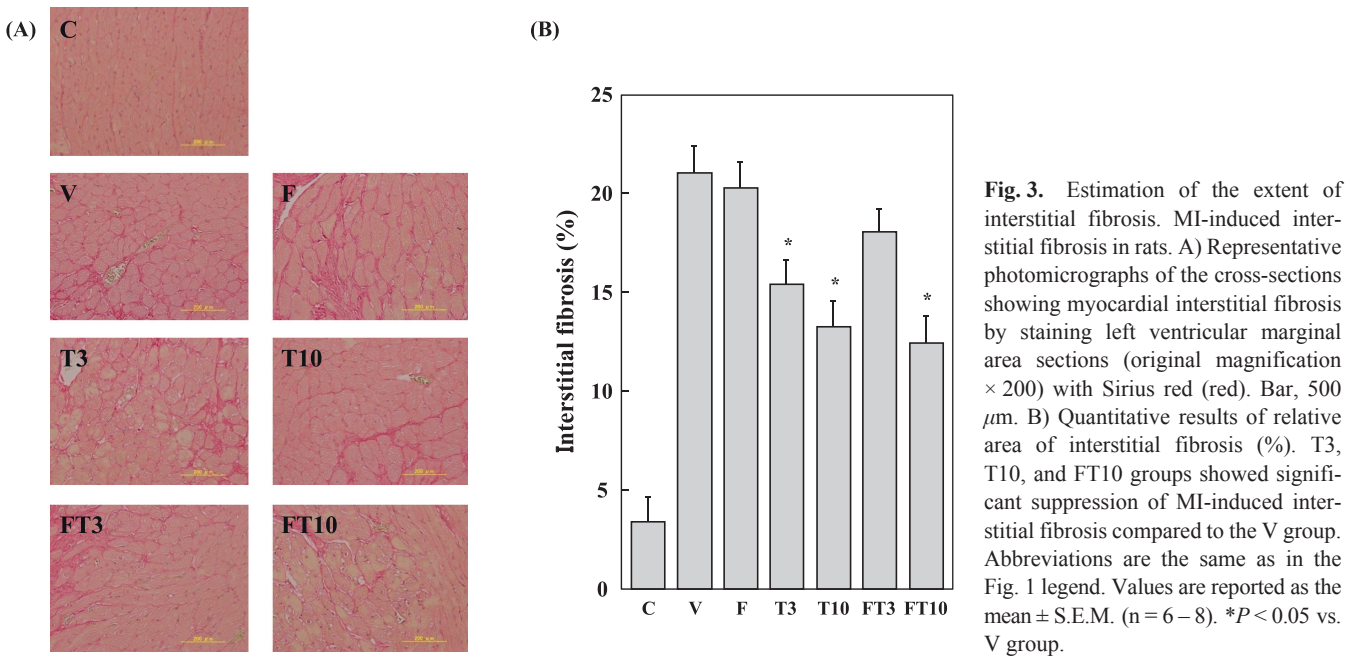

Fig. 3. Estimation of the extent of interstitial fibrosis. MI-induced interstitial fibrosis in rats. A) Representative photomicrographs of the cross-sections showing myocardial interstitial fibrosis by staining left ventricular marginal area sections (original magnification × 200) with Sirius red (red). Bar, 500 μm. B) Quantitative results of relative area of interstitial fibrosis (%). T3, T10, and FT10 groups showed significant suppression of MI-induced interstitial fibrosis compared to the V group. Abbreviations are the same as in the Fig. 1 legend. Values are reported as the mean ± S.E.M. (n = 6 – 8). **P* < 0.05 vs. V group.

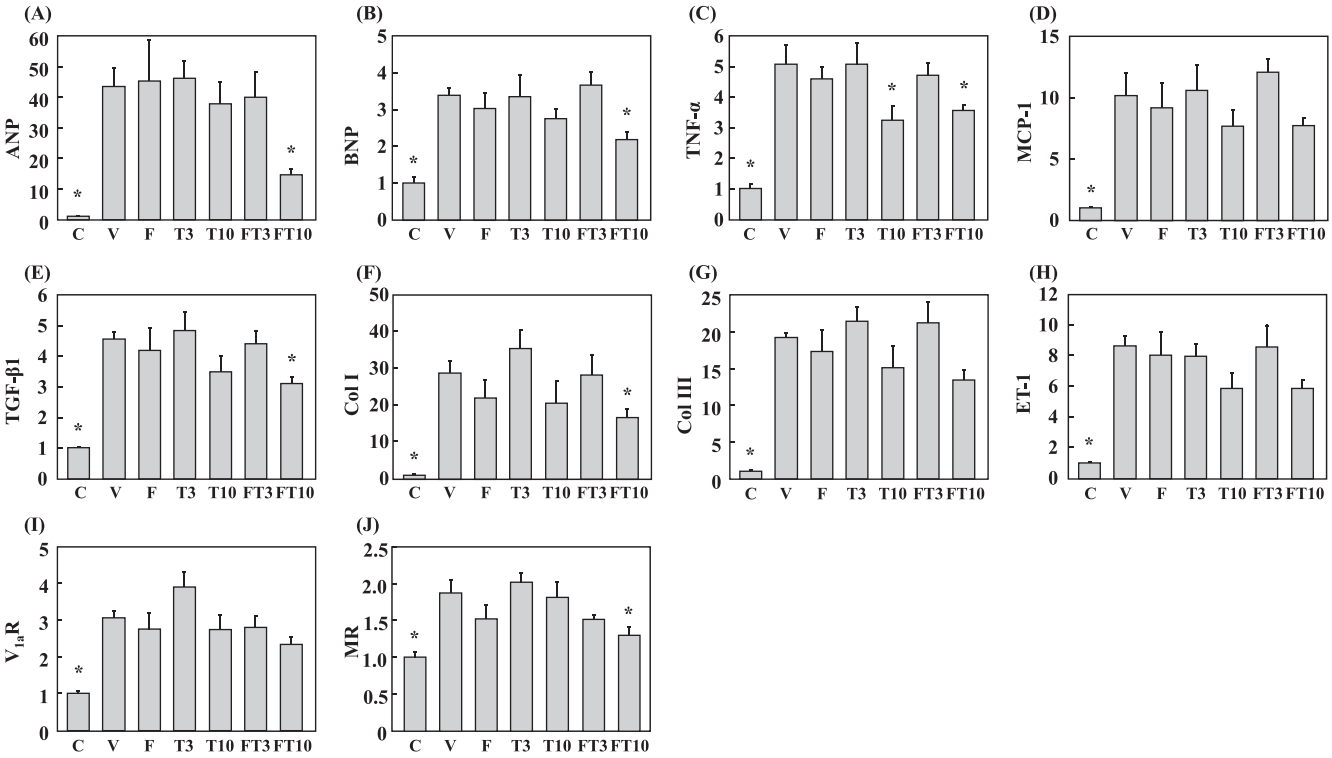

Fig. 4. mRNA expression. Gene expression in the marginal infarct region of the vehicle-treated and medicated rats and left ventricle of the control rats. The bar graph shows the value of each mRNA, corrected for the GAPDH mRNA value. Mean values in the control rat group are represented as 1. All parameters of mRNA expressions were up-regulated by MI induction. A, B) TNF-α mRNA expression was significantly decreased in the T10 and FT10 groups. MCP-1 mRNA expression did not show any significant difference. C, D) The MI-induced up-regulation of ANP and BNP was significantly decreased in the FT10 group. E, F, G) TGF-β1 and Col-I mRNA expressions were significantly decreased in the FT10 group. Col III mRNA expression did not show any significant difference. H, I) MI-induced ET-1 or $V_{1a}R$ mRNA expression was not significantly decreased in any of the treated groups. J) MI-induced MR mRNA expression was significantly decreased in the FT10 group. ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor-β1; Col III, collagen type III; ET-1, endothelin-1; $V_{1a}R$, vasopressin V_{1a} receptor; MR, mineralocorticoid receptor. Other abbreviations are the same as in the Fig. 1 legend. Values are reported as the mean ± S.E.M. (n = 6 – 8). *$P < 0.05$ vs. V group.

over, the LVEF in the T10, FT3, and FT10 groups significantly improved compared to that of the V group (Fig. 2C).

Estimation of interstitial fibrosis in LV

The extent of interstitial fibrosis in the marginal zone of the infarct is shown in Fig. 3. The extent of interstitial fibrosis was significantly lower in the T3, T10, and FT10 groups (15.3% ± 1.2%, 13.2% ± 1.2%, and 12.4% ± 1.3%, respectively) than in the V group (21.0% ± 1.3%), while this effect was not shown in the F group (20.2% ± 1.3%).

Effect of tolvaptan on cardiac gene expression

Expression of mRNA in the LV marginal area in each group was measured by qRT-PCR (Fig. 4). The mRNA expression of atrial natriuretic peptide (ANP) and BNP, which are closely associated with cardiac load, increased by 43.4- and 3.4-fold, respectively, because of MI induction ($P < 0.01$). Up-regulation of ANP or BNP expression by MI significantly decreased in the FT10 group.

MI-induced a 5.1- and 10.2-fold ($P < 0.05$) increase in the mRNA expression of tumor necrosis factor (TNF)-α and MCP-1, respectively. TNF-α and MCP-1 are associated with cardiac inflammation. This increase in TNF-α expression was significantly suppressed in the T10 and FT10 groups.

The mRNA expression of TGF-β1 and collagen type I and III (Col-I and Col-III), which are closely associated with cardiac fibrosis, increased by 4.6-, 28.8-, and 19.2-fold, respectively, because of MI induction ($P < 0.05$). Up-regulation of TGF-β1 or Col-I was significantly inhibited in the FT10 group.

In addition, the mRNA expressions of the $V_{1a}R$ and endothelin-1 (ET-1), which are closely associated with vasoconstriction and neurohumoral factors, significantly increased because of MI induction; however, none of the treatments attenuated the expression of these two genes.

Lastly, we measured the mineralocorticoid receptor (MR) gene expression in the rat myocardium to investigate the effect of tolvaptan on cardiac RAAS. MI-induced MR expression was significantly decreased in the FTH group.

Discussion

The present study revealed that tolvaptan could prevent the progression of cardiac remodeling in the acute phase following MI. The combination therapy of furosemide and tolvaptan may be useful for acute HF.

Acute congestive HF is a common clinical syndrome in acute MI, and loop diuretics are often used to improve pulmonary congestion. Although continuous furosemide injection induces a sustained reduction in preload in acute MI patients without overt HF, this has been associated with potentially disadvantageous hemodynamic findings (19). However, we are not aware of any evidence demonstrating a protective effect and positive prognosis associated with furosemide administration at the time of acute MI. Tolvaptan was developed as a new diuretic drug that exerts an aquaretic effect by blocking the V_2R at the renal collecting duct, thereby inhibiting water reabsorption (6, 10).

Several clinical studies such as EVEREST and the Effect of Tolvaptan on Hemodynamic Parameters in Subjects with Heart Failure (ECLIPSE) trial have shown that tolvaptan increases urine output in a dose-dependent manner in the case of chronic HF, but has no significant effect on the secondary endpoints of BP, HR, systemic vascular resistance, and cardiac index (11, 13, 20 – 22). However, it was previously thought to be impossible to elucidate whether tolvaptan could independently improve LVEF in chronic HF patients, because most of these patients were concomitantly receiving other medication. We recently investigated the efficacy of furosemide, tolvaptan, and a furosemide-tolvaptan combination therapy for chronic HF with old MI in rats; our evidence suggested that tolvaptan improved LV systolic and diastolic function (14). The area of interstitial fibrosis and infiltration of macrophages were inhibited in the marginal area of the infarction. The mRNA expression of MCP-1 and TNF-α, which are inflammatory markers, and TGF-β1 and Col III, which are fibrosis markers, were suppressed in the marginal area of the MI. Moreover, tolvaptan suppressed the mRNA expression of $V_{1a}R$ and ET-1. These results could be expected to reduce cardiac load and may reflect the pleiotropic effects of tolvaptan. Unfortunately, the cardioprotective effects of tolvaptan in acute HF remain unknown.

To our knowledge, the present study is the first attempt to evaluate the effects of tolvaptan administration alone in acute MI. LVEF in the groups treated with high-dose tolvaptan or a combination of furosemide and tolvaptan was greater than that in the groups treated with the vehicle or furosemide alone. Tolvaptan alone or the combination of furosemide and high-dose tolvaptan significantly attenuated MI-induced interstitial fibrosis. Furthermore, high-dose tolvaptan down-regulated the mRNA expression of fibrotic mediators such as TGF-β1 and Col I in the marginal area of the MI. These results are not contradictory to those of our previous report, in which a model of chronic HF was described (14). Our previous study suggests that tolvaptan may have an anti-inflammatory action that inhibits macrophage infiltration and mRNA expression of MCP-1 in the marginal area of infarct myocardium in the chronic HF model, although there were no significant changes in mRNA expression of MCP-1 in this acute phase following MI. It is difficult to explain these contradictory results; however, the insufficiency to reduce inflammation in this study may be attributable to the strong inflammatory reaction associated with the acute phase of MI and the short medication period.

TGF-β1 is a locally generated cytokine that has been implicated as a major contributor to tissue fibrosis in various organ systems (23). Previous studies have shown that the expression of TGF-β1 mRNA is increased in the LV myocardium of patients with idiopathic hypertrophic cardiomyopathy and dilated cardiomyopathy and in animal models of hypertension, myocardial infarction, and pressure overload (17, 24 – 25). TGF-β1 expression is elevated in hypertrophic myocardium during the transition from stable hypertrophy to HF in both experimental models and human HF, and it is one of only a few markers discriminating compensated cardiac hypertrophy from decompensated cardiac hypertrophy (25). Thus, TGF-β1 may be responsible for the development of LV remodeling. In our study, TGF-β1 mRNA expression and interstitial fibrosis in the FT10 group were significantly reduced, compared with those in the V group.

It is difficult to determine whether the anti-fibrotic effect of tolvaptan is a direct effect. Tolvaptan is pharmacologically classified as a V_2R antagonist (6); however, V_2R is not expressed in the myocardium. We did not detect LV V_2R expression in this study (data not shown). However, our study showed that the ventricular weight and LVEF were significantly improved in the

group treated with a combination of furosemide and high-dose tolvaptan. In addition, since the groups did not differ in terms of BW, the effect of tolvaptan may not be entirely explained by its volume-reducing effect. Recent studies conducted in our laboratory and by other researchers have reported that tolvaptan suppressed $V_{1a}R$ activation of the myocardium in a model of hypertensive HF and in a model of MI-induced chronic HF (14, 26). The results from these studies also suggested that the underlying mechanism might be related to the suppression of neurohumoral activation. Unfortunately, in the present study, expression of neither $V_{1a}R$ nor ET-1 mRNA in the LV was attenuated by tolvaptan, although the combination of tolvaptan and furosemide tended to attenuate expression of these mRNAs. The reason may be that the changes of acute phase after MI are very strong.

Previous studies have shown that furosemide increases PRA levels, while administration of the combination of furosemide and tolvaptan does not increase PRA (3, 27). Moreover, tolvaptan promotes the removal of excess water from the body without activating the RAAS or causing serum electrolyte imbalance (28). In the present study, the effect of tolvaptan on the circulating RAAS was unclear because neither PRA nor PAC was elevated by MI induction. At the same time, furosemide alone or tolvaptan alone elevated PAC. Interestingly, the combination of furosemide and tolvaptan did not raise it. Furthermore, the combination of furosemide and tolvaptan significantly inhibited MI-induced MR expression in the myocardium, suggesting that the combination of furosemide and tolvaptan may repress not circulating but cardiac RAAS activity.

It is difficult to measure AVP level because of considerable technical challenges related to AVP's short plasma half-life, interaction with platelets in the serum, and small size. Copeptin is a stable peptide derived from the precursor of AVP and a sensitive surrogate marker for AVP release (29 – 30). In this study, MI significantly elevated plasma copeptin level, and tolvaptan alone did not change the level. Interestingly, furosemide alone or the combination of furosemide and tolvaptan inhibited the copeptin level. These results suggest that the combination therapy of furosemide and tolvaptan could have more relevant effect than a single therapy for preventing MI-induced cardiac remodeling.

Study limitations

The observations in the present study do not show whether the combination therapy of furosemide and tolvaptan has a clinically more relevant effect to the patients with acute MI. The effect of tolvaptan on cardiac remodeling after ischemia/reperfusion model has not been examined because the MI model in the present study is a permanent ligation model. The effect of tolvaptan on sympathetic nervous system remains unexplained. Therefore, further studies are needed to elucidate the precise mechanism of the effects of tolvaptan or the synergic effect of the combination therapy in acute HF.

In conclusion, our present study provides the first in vivo evidence showing the beneficial effects of tolvaptan in acute MI. This effect may be attributed to its volume-reducing effect as well as to its anti-fibrotic actions. We propose the combination treatment of furosemide and tolvaptan would be a new therapy for acute HF after MI.

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Conflicts of Interest

None declared.

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