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Review

New therapeutic target for the non-electrophysiological signaling in atrial fibrosis and fibrillation such as inflammation

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ABSTRACT

We have experimentally established appropriate models of atrial fibrillation (AF) with atrial interstitial fibrosis. Two approaches were adopted. Firstly, left atrial fibrosis was induced by continuous infusion of angiotensin II (AII). In an electrophysiological study using isolated perfused heart, AF was easily induced following AII treatment. Repeated whole-body hyperthermia led to the induction of heat-shock protein 72, which resulted in attenuation of AII-induced left atrial fibrosis and suppression of AF inducibility. Secondly, atrial fibrosis was induced by pressure overload by abdominal aortic constriction (AAC). AAC enhanced left atrial expression of monocyte chemoattractant protein-1 and activity of matrix metalloproteinase-9. Treatment with pioglitazone, a peroxisome proliferator-activated receptor-γ agonist, resulted in attenuation of pressure overload-induced left atrial fibrosis and suppression of AF inducibility. In the same AAC model, the effects of candesartan on gap junction remodeling were investigated. Connexin 43 (Cx43) of the left atria was firmly located in the intercalated disks in control rats. A progressive redistribution of Cx43 from the intercalated disk to the lateral surface (lateralization) was observed in AAC rats. Candesartan prevented left Cx43 lateralization. Thus, heat-shock proteins, pioglitazone, and candesartan could be novel therapeutic approaches to prevent atrial fibrosis and AF.

Contents

1. Introduction

Atrial fibrillation (AF) is the most common type of arrhythmia observed in a clinical setting, and is associated with significant morbidity and mortality [1,2]. The "downstream" approach that targets ion channels using antiarrhythmic drugs, has shown to be limited because antiarrhythmic drugs are ineffective in about half of the patients and often induce adverse effects, including proarrhythmia [3]. However, the "upstream" approach that targets processes involved in the development of the substrates that promote AF, has recently attracted much attention [4]. An atrial tachypacing-induced AF model has been established to represent clinical AF,[5] which is characterized by shortening of the atrial effective refractory period (ERP). Interestingly, Kumagai et al. [6] reported that in a canine AF model with rapid atrial pacing at 400 beats/min for 5 weeks, rather than shortening the atrial ERP, extensive interstitial fibrosis was found in the atrial free wall in

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association with gradual conduction prolongation in the atria. This might be the first report to show "structural remodeling" by atrial tachypacing [6]. However, in patients without structural heart disease, the atrial tachypacing procedure that is followed in "normal" animals may be applicable only by perpetuation of paroxysmal AF into permanent AF.

Therefore, we attempted to induce atrial fibrosis with enhanced AF vulnerability using continuous angiotensin II (AII) infusion [7] or pressure overload by abdominal aortic constriction (AAC) [8]. These procedures are more likely to correlate with the mechanisms underlying newly developed AF in patients with lifestyle diseases, such as hypertension. Many review articles in terms of upstream therapies for AF have been published elsewhere [9–11]. Therefore, in this special review article, we introduce our experimental approaches for the prevention of atrial fibrosis.

2. AF model by continuous infusion of angiotensin II

2.1. Protective effects of heat-shock proteins

The heat-shock proteins (HSPs) are an important family of endogenous protective proteins that increase in response to a wide variety of stresses, such as heat shock, hypoxia, hydrogen peroxide, inflammation, and ischemia [12]. We initially showed that oral geranylgeranylacetone (GGA) is cardioprotective against ischemic insult via its induction of HSP72 [13]. Mandal et al. [14] showed that in patients undergoing elective coronary artery bypass surgery, the preoperative HSP72 content in right atrial tissue obtained at surgery was higher in patients who did not develop postoperative AF than in those who did develop AF. Therefore, we tested the hypothesis that atrial fibrosis and AF evoked by AII could be prevented by the induction of HSP72 [7]. By using cultured rat left atrial fibroblasts, we showed that pretreatment with hyperthermia (42 °C for 30 min) induced HSP72 expression, peaking at 8 h following the application of hyperthermic conditions (Fig. 1A). In fibroblasts treated under hyperthermic conditions, AII-induced extracellular signal-regulated kinase (*Erk*1/*Erk*2) phosphorylation (Fig. 1B), α-smooth muscle actin (α-SMA) expression (Fig. 2), transforming growth factor-β1 (TGF-β1) secretion, collagen synthesis, and the expressions of collagen type-1 and tissue inhibitor of metalloproteinases-1 were attenuated. A small interfering RNA targeting HSP72 could abolish the anti-fibrotic effects of hyperthermia (Figs. 1 and 2). Therefore, we concluded that HSPs, particularly induction of HSP72, have a dominant role in the suppression of AII-induced fibrotic signal [7]. In addition, in experiments in vivo, repeated hyperthermia (43 °C for 20 min) prevented induction of left atrial interstitial fibrosis by continuous infusion of AII (Fig. 3). In an electrophysiological study using isolated perfused heart continuous AII infusion caused slowing of interatrial conduction without affecting atrial refractoriness. In AII-treated heart, extrastimuli from the right atrial appendage resulted in a high incidence of repetitive atrial responses (RARs). These were suppressed by hyperthermia treatment, resulting in reduced inducibility of RARs by extrastimuli (Fig. 4) [7]. On the basis of these observations, we concluded that hyperthermia treatment is effective in suppressing AII-mediated atrial fibrosis and AF via, at least in part, induction of HSP72 [7].

On the other hand, several studies indicated that HSP27, a small-sized HSP, may play a particularly important role in AF pathogenesis [15,16]. Using HL-1 myocytes derived from mouse atria, Brundel et al. [15] showed that tachypacing-induced myolysis was prevented by hyperthermia pretreatment or GGA pretreatment. They found that HSP27, but not HSP72-transfection, was sufficient for protection against tachypacing-induced myolysis. Brundel et al. [16] subsequently showed that in HL-1 cells, tachypacing-induced reduction in duration of L-type Ca^{2+} current and action potential was prevented by GGA treatment via induction of HSP27. They also showed that in dogs in vivo, atrial tachypacing shortened the atrial ERP, which was attenuated by administration of GGA. GGA also suppressed tachypacing induced AF-promoting changes, including AF duration by burst pacing and AF vulnerability [16]. Although the effects of HSP27 on atrial fibrosis remain unclear, these observations suggest that HSP induction particularly that of HSP27, may be an effective approach to prevent the progression of clinical AF.

Coronary artery disease is associated with an increased risk of AF [17]. Sinno et al. [18] reported that in dogs, experimental atrial ischemia resulted in slow conduction, which stabilized the re-

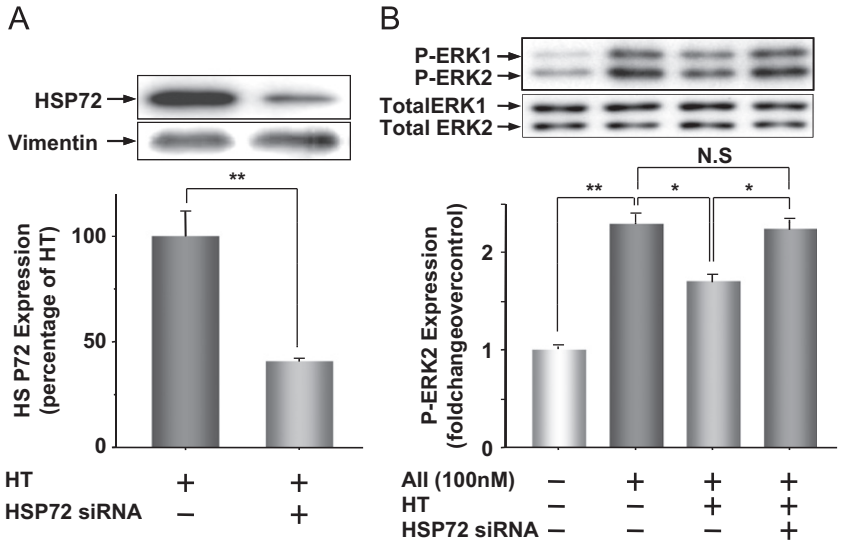

Fig. 1. A: Expression of heat-shock protein 72 (HSP72) in atrial fibroblasts analyzed by western blot. Above: representative bands of HSP72 at 8 h after treatment under hyperthermic (HT) conditions in the absence (left) and presence of HSP72 siRNA (right). Vimentin was used as an internal standard. Below: quantitative expression of HSP72 (relative density). B: Extracellular signal-regulated kinase 1/2 (ERK1/ERK2) phosphorylation in atrial fibroblasts analyzed by western blot. Above: representative bands of phosphorylated-ERK 1/2 (P-ERK 1/2; top) and total ERK 1/2 (bottom). Below: quantitative expression of P-ERK2 (relative density). Data are mean ± standard error of the mean (SEM). In each experiment (A, B, C), 4 independent cultures were evaluated. $^{*}p < 0.05$, $^{**}p < 0.01$, NS = not significant. (Figure adapted with permission (7)).

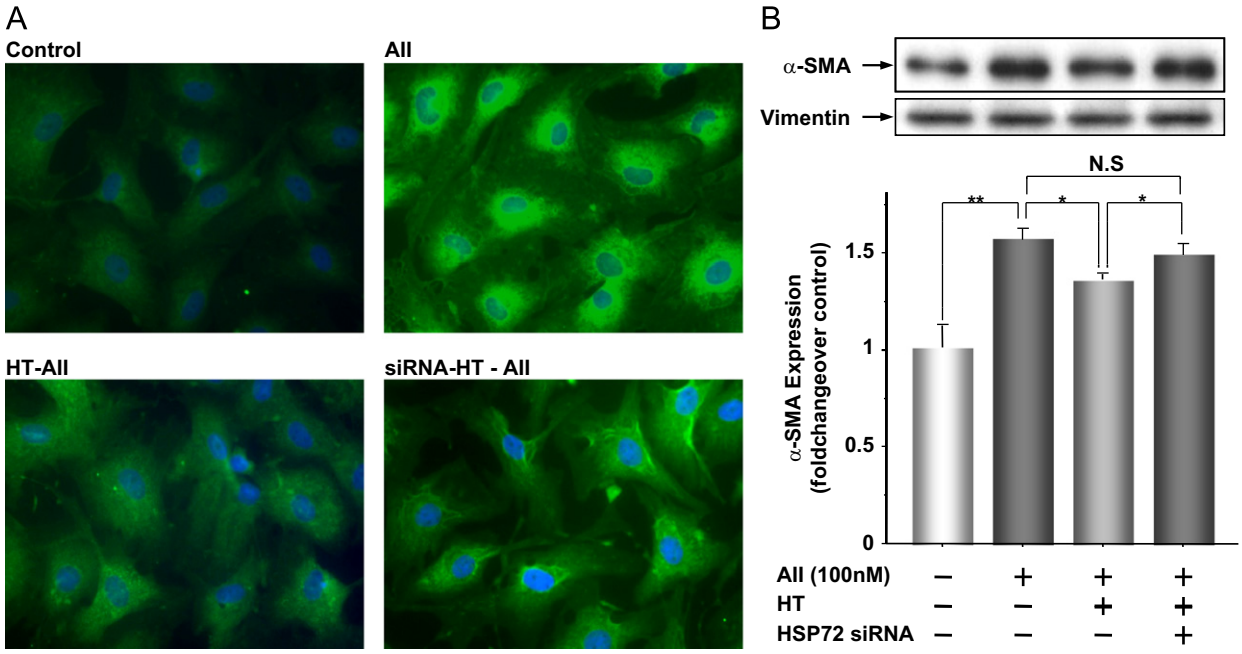

Fig. 2. Expression of α-smooth muscle actin (α-SMA) in atrial cardiac fibroblasts. A: Immunocytochemical staining of atrial fibroblasts (passage 2) using specific α-SMA primary antibodies. B: Above: representative bands of α-SMA by western blot analysis. Below: quantitative expression of α-SMA protein (relative density). AII = angiotensin II. HT = hyperthermia. Data are mean ± standard error of the mean (SEM). Four independent cultures were evaluated. $^{*}p < 0.05$, $^{**}p < 0.01$, NS = not significant. (Figure adapted with permission (7)).

entrant AF circuit. Subsequently, by using the same atrial ischemia dog model, Sakabe et al. [19] reported that HSP72 induction by orally administered GGA suppressed conduction heterogeneity and burst pacing-induced AF duration. In the ischemic and non-ischemic regions, HSP72 was intensively induced by GGA, whereas HSP27 induction was not significant. Studies from our research group showed that HSP72 induction attenuated ventricular ischemia/reperfusion injury [13,20–22]. Thus, HSP72 rather than HSP27 appears to have a more important role in the prevention of ischemia-induced production of AF substrate.

Thus, experimental studies have shown that atrial tachypacing, continuous exposure to AII, or acute atrial ischemia causes electrical and structural remodeling. This is characterized by shortening of the atrial ERP and fibrosis-mediated conduction abnormality, leading to reduction in wavelength, which promotes AF development (Fig. 5) [23]. Induction of heat shock responses has been shown to protect the heart against AF by preventing electrical and structural remodeling. The potent protective role of heat-shock responses against the progression of clinical AF has been clarified [24]. Recently, serum HSP27 levels were reportedly correlated with left atrial dimension, left atrial voltage, and fractionated intervals, and predicted AF recurrence after catheter ablation [24]. Baseline serum level of HSP27 also correlated with interleukin-10 and tumor necrosis factor-α (TNF-α) levels, which showed that the protective mechanisms of HSP27 against AF could be related to inflammation [24]. Taken together, interventions that induce heat shock responses may prevent newly developed AF and delay progression of paroxysmal AF to persistent AF.

The significant therapeutic potential of HSP72 for Duchenne muscular dystrophy (DMD) was recently shown [25]. In *mdx* and *dko* mice, phenocopies of DMD, the function of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) showed severe dysfunctioning. However, BGP-15, a pharmacological inducer of HSP72, preserved SERCA function and decreased muscle degradation in association with HSP72 upregulation [25]. The ability of HSP72 to maintain intracellular Ca^{2+} homeostasis may prevent AF, especially where SERCA dysfunction is involved [26].

3. AF model by pressure overload (abdominal aortic constriction)

3.1. Protective effects of pioglitazone against inflammatory profibrotic signals

Pioglitazone, a peroxisome proliferator-activated receptor γ (PPARγ) agonist, possesses anti-inflammatory properties [27]. Pioglitazone has been shown to attenuate left ventricular hypertrophy and fibrosis in salt-sensitive hypertensive rats [28,29]. However, the anti-profibrotic effects of pioglitazone in the atria have been poorly investigated [30]. We therefore tested the hypothesis that atrial fibrosis and enhanced vulnerability to AF evoked by pressure overload could be attenuated by pioglitazone via suppression of inflammatory profibrotic signals [8]. Rats were subjected to AAC. Pioglitazone (3 mg kg^{-1} day^{-1}) or vehicle was orally administered for 4 weeks. We observed the following. AAC enhanced the protein expression of monocyte chemoattractant protein (MCP)-1 (Fig. 6A), TGF-β1 (Fig. 6B), and α-SMA in the left atrium. Messenger RNA expression of collagen type 1 and atrial natriuretic peptide in the left atrium was increased by AAC. Gelatin zymography showed that the activity of promatrix metalloproteinase-9 was increased by AAC (Fig. 7). AAC induced left atrial fibrosis (Fig. 8). In isolated-perfused heart experiments, AAC did not alter the refractory period of the left atrium or the right atrium, but it did prolong the inter-atrial conduction time. Programmed extrastimuli from the right atrium induced AF in all of the AAC-treated rats. All of these changes induced by AAC were suppressed by treatment with pioglitazone (Figs. 6–8). Evidence has shown that inflammation contributes greatly to the progression of atherosclerosis [31]. The process of atrial structural remodeling observed in AF has been revealed to mimic that of atherosclerosis [32]. In atherosclerosis, MCP-1 plays an

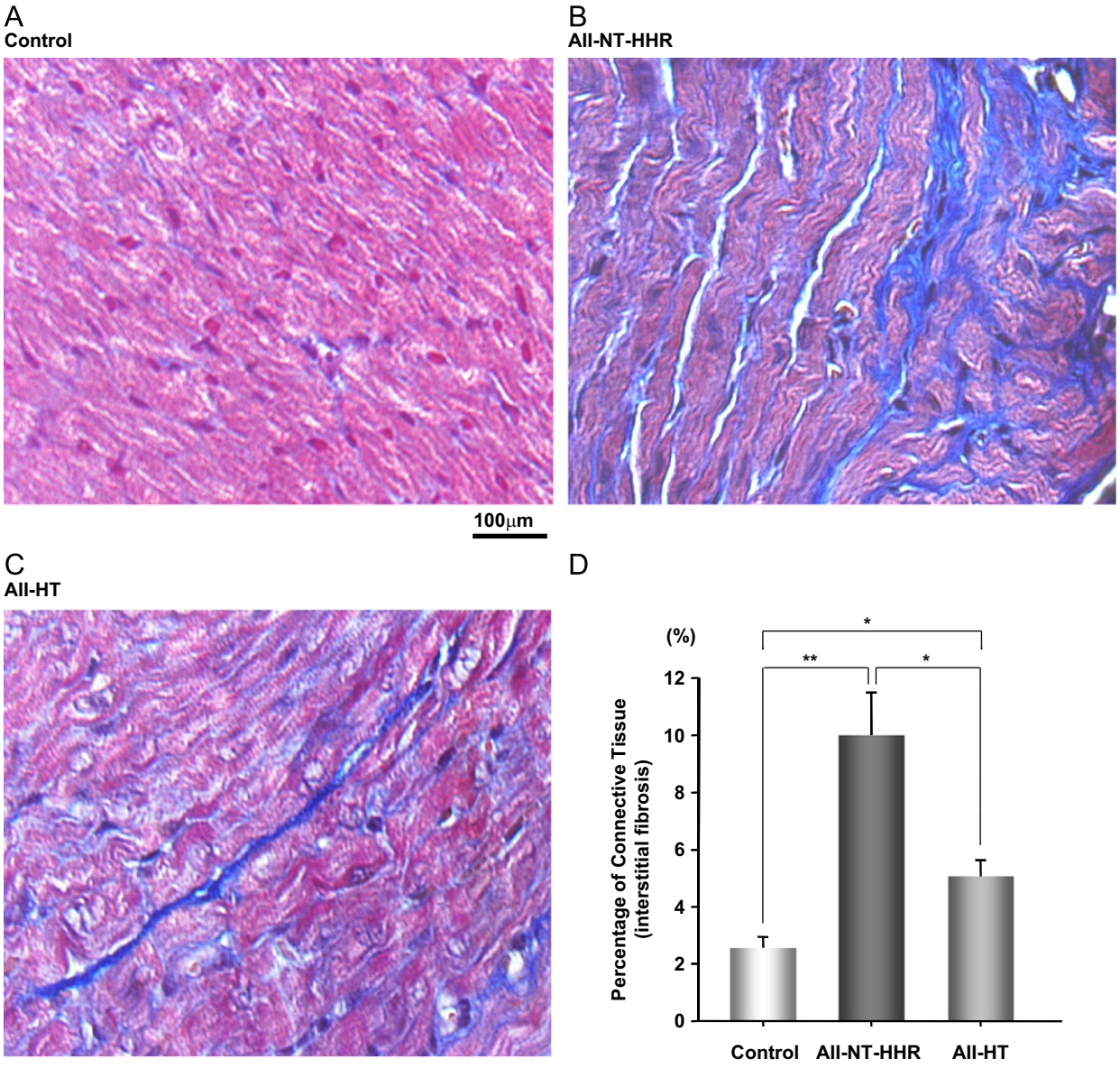

Fig. 3. Histology of the left atrial (LA) free wall. A–C: Representative atrial tissue sections stained by Masson trichrome staining. D: Histogram of mean interstitial fibrosis of the LA in the control, angiotensin II (AII)-hyperthermia (HT) and AII-NT-hydralazine, hydrochrolothiazide, and reserpine (HHR) groups. Data are mean ± standard error of the mean (SEM),. $n=7–8$ in each group. (Figure adapted with permission (7)).

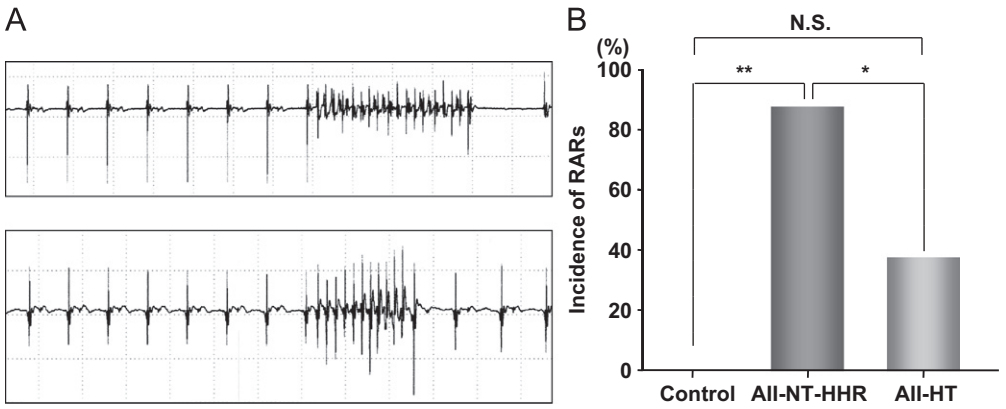

Fig. 4. Repetitive atrial response (RAR). A: Two representative RAR observed in the angiotensin II (AII)–NT-hydralazine, hydrochrolothiazide, and reserpine (AII-NT-HHR) group. B: Incidence of RAR. $^{*}p<0.05$, $^{**}p<0.01$, NS=not significant. (Figure adapted with permission (7)).

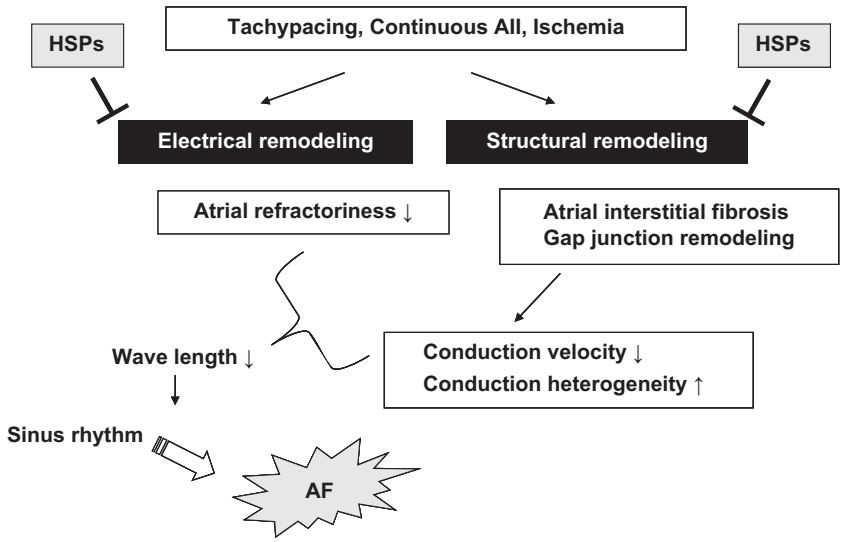

Fig. 5. Tachypacing, continuous infusion of angiotensin II (AII), and ischemia induce electrical remodeling and structural remodeling in atria. This results in a reduction in wavelength, which is a substrate for the development and progression of atrial fibrillation (AF). Induction of heat-shock proteins (HSPs) can prevent atrial remodeling. (Figure adapted with permission (23)).

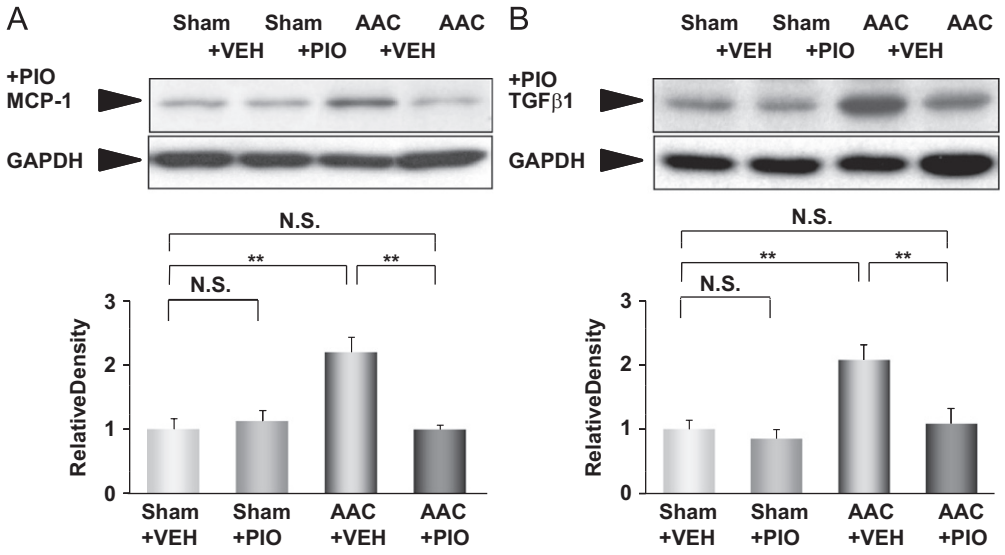

Fig. 6. Expression of monocyte chemoattractant protein (MCP)-1 and transforming growth factor-β1 (TGFβ1) in left atrial tissue. A: Expression of MCP-1 in left atrial tissue analyzed by western blot. Top: representative bands of MCP-1. Bottom: quantitative expression of MCP-1 (relative density). B: Expression of TGF-β1 in left atrial tissue analyzed by western blot. Top: representative bands of TGF-β1. Bottom: quantitative expression of TGF-β1 (relative density). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal standard. Data are mean ± standard error of the mean (SEM) and are expressed as relative to an average of the Sham+vehicle (VEH) groups. $n=5$ in each group. $^{**}p < 0.01$, N.S.=not significant. AAC=abdominal aortic constriction; PIO=pioglitazone. (Figure adapted with permission (8)).

early and important role in the recruitment of monocytes to atherosclerotic lesions, and in the formation of intimal hyperplasia after arterial injury [33]. Such early inflammatory cell infiltration of injured vessel surface was previously reported to be suppressed by pioglitazone, in association with the reduction of activated MCP-1 expression [34]. In a pressure-overloaded ventricle perivascular macrophage infiltration, mediated by MCP-1, provokes interstitial fibrosis via production of inflammatory cytokines, such as TGF-β1. Blocking MCP-1 inhibited TGF-β1 induction [35,36]. Taken together with our observations, [8] it is conceivable that MCP-1 is a key molecule in AAC-induced inflammatory fibrotic processes in the atria and pioglitazone attenuates atrial fibrosis, probably via suppression of MCP-1 expression. Yamashita et al. [32] showed that MCP-1 in a left atrial appendage obtained from patients undergoing cardiac surgery, was significantly higher in patients with AF than in those with sinus rhythm. Li et al. [37] studied the serum concentration of MCP-1 in 350 AF patients and 150 control patients and also reported that serum MCP-1 levels were almost twice as high in AF patients than in controls.

The anti-AF effects of pioglitazone have been poorly investigated. Shimano et al. [30] reported that pioglitazone reduced the durations of induced AF, attenuating atrial fibrosis, and reducing interatrial conduction time, by using rapid ventricular pacing-induced congestive heart failure (CHF) in rabbits. Their observations are generally consistent with that of our study [8]. Atrial profibrotic processes provoked by both CHF and pressure overload appeared to share common molecular signatures, at least in part, and they can be suppressed by pioglitazone. More recently, Xu et al. [38] showed that pioglitazone prevents age-related atrial remodeling and AF promotion and that its inhibitory effect is partially attributable to increases in HSP72 expression and antioxidant activity. Apoptotic cell death and cell survival were also improved by pioglitazone [38]. These observations suggest that

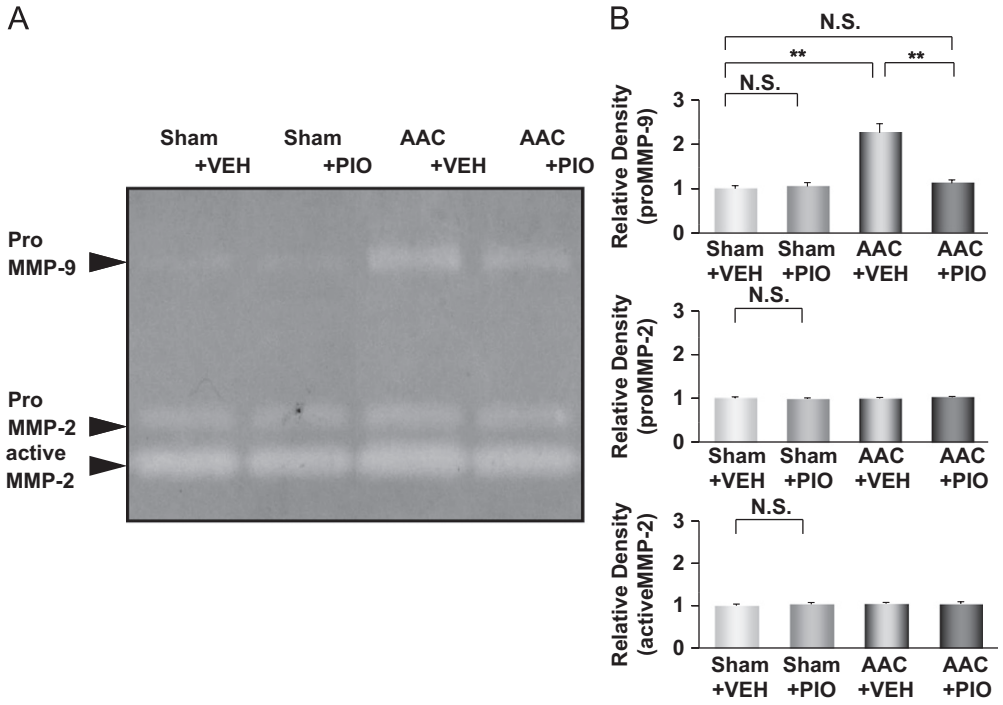

Fig. 7. Expression of matrix metalloproteinases (MMP)-2 and -9 in left atrial tissue was analyzed using gelatin zymography. A: Representative bands of MMP-2 and MMP-9. B: Quantitative expression of MMP-2 and MMP-9 (relative density). Data are mean ± standard error of the mean (SEM), and are relative to an average of the Sham+vehicle (VEH) samples. $n=5$ in each group. $^{**}p < 0.01$, N.S.=not significant. (Figure adapted with permission (8)).

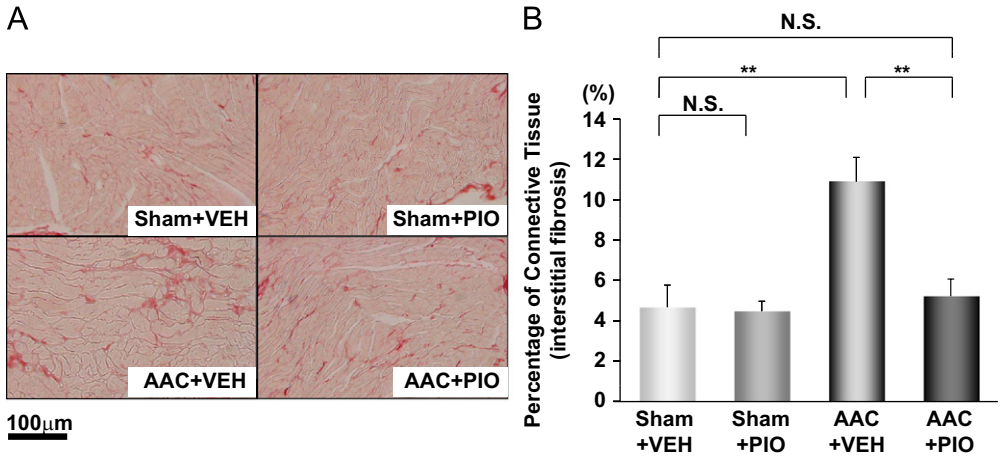

Fig. 8. Left atrial fibrosis. A: Representative Sirius-red staining of 4 experimental groups. B: The mean percentage of interstitial fibrosis of the left atrium in each group. Data are mean ± standard error of the mean (SEM). $n=5$ in each group. $^{**}p < 0.01$, N.S.=not significant. (Figure adapted with permission (8)).

inflammatory profibrotic mechanisms are involved in AF vulnerability in AF models by pressure overload, CHF, and aging. Pioglitazone is expected to be effective at attenuating atrial fibrosis, possibly via suppression of inflammatory profibrotic processes. Gu et al. [39] conducted a prospective observational cohort study of 150 consecutive patients undergoing catheter ablation of drug-refractory paroxysmal AF, who had a history of type 2 diabetes mellitus. Pioglitazone improved the preservation of sinus rhythm and reduced the reablation rate [39].

3.2. *Protective effects of candesartan against gap junction remodeling*

Experimental studies have shown the beneficial effects of AII type-1 (AT1) receptor blockers (ARB) in suppression of atrial fibrosis and AF. For example, treatment with candesartan effectively suppressed fibrosis and AF in a canine model of AF with rapid atrial pacing for 5 weeks, in which extensive interstitial fibrosis was found in association with a gradual conduction prolongation in the atria [6]. In addition to fibrosis, remodeling of the gap junction due to various etiologies has been recognized in AF [40]. The predominant gap junction proteins expressed in the atria are connexins 40 and 43 (Cx40 and Cx43) [41–43]. A nonsense mutation in Cx40 has been found in patients with familial AF [41]. In addition, redistribution of Cx43 has been shown to be involved in abnormal conduction, leading to AF maintenance [43,44]. We therefore investigated the effects of candesartan on atrial fibrosis, Cx43 remodeling and enhanced AF vulnerability induced by pressure overload (unpublished data). AAC rats were treated with a subdepressor dose of candesartan (0.1 mg kg^{-1} day^{-1}, p.o.) for 4 weeks. The Cx43 of left atrium was firmly located in the intercalated disks in the control group, and

did not redistribute during the 4-week observation period. Representative and quantitative estimation of Cx43 distribution in the AAC-treated rat left atrium are demonstrated in Fig. 9. Seven days after the AAC procedure, there was a tendency to redistribute Cx43 from the intercalated disk to the lateral surface (lateralization). Compared to day 0, on days 14 and 28 after AAC, significant lateralization of Cx43 was quantitated and documented. The Cx43 lateralization induced by AAC was attenuated by treatment with

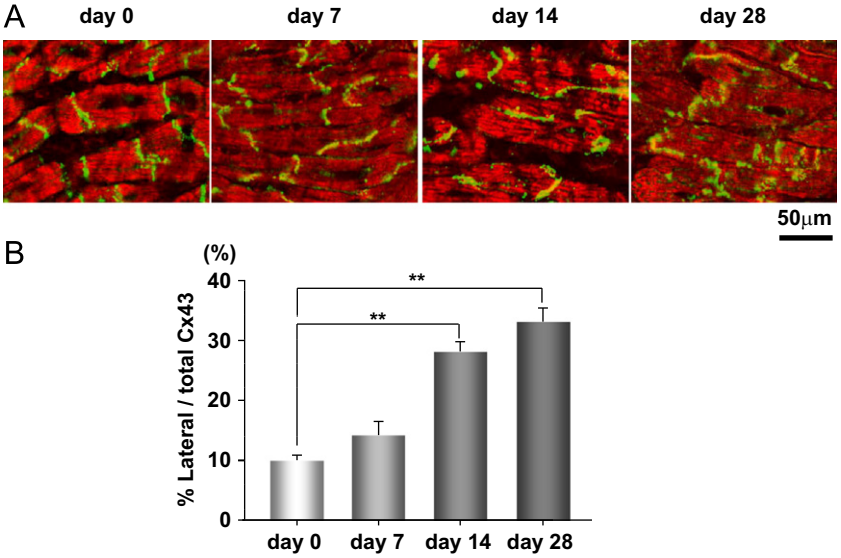

Fig. 9. Time course of left atrial (LA) connexin 43 (Cx43) distributions in rats subjected to abdominal aortic constriction (AAC) with treatment of vehicle (AAC-VEH group). Rat heart was isolated before aortic constriction (AC; day 0) and on days 7, 14, and 28 after AC. A: Representative immunohistochemical staining of LA using specific Cx43 primary antibodies. B: Quantitative analysis of Cx43 lateralization. Data are mean ± standard error of the mean (SEM). $n=8$ in each group. $^{**}p < 0.01$.

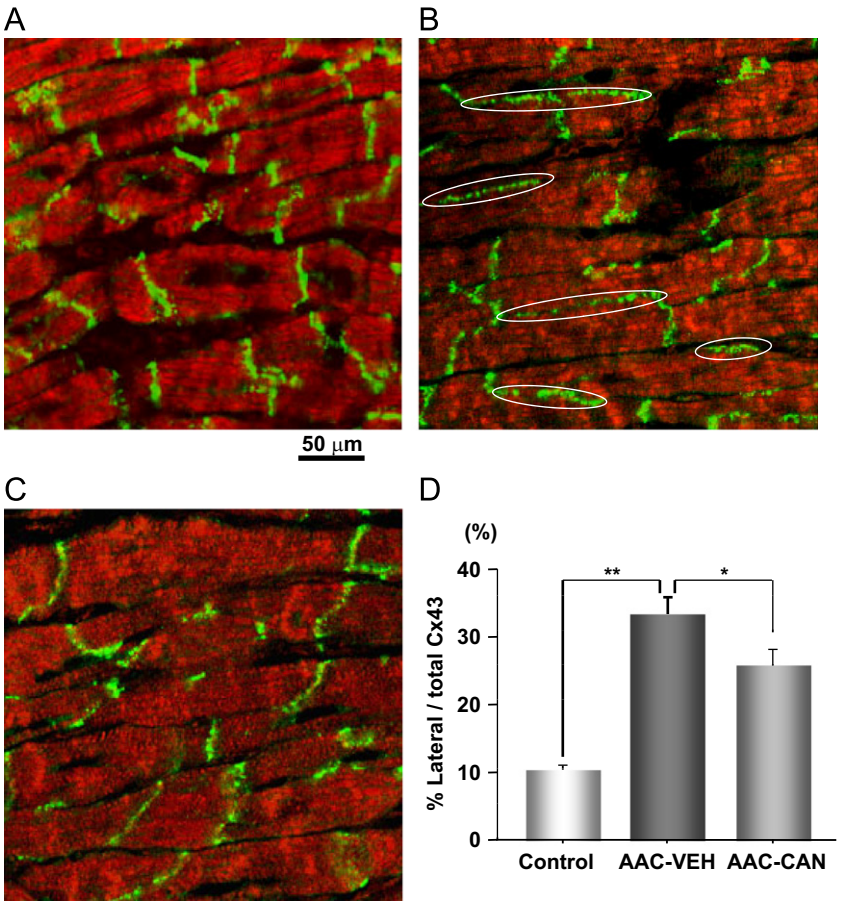

Fig. 10. Effects of candesartan (CAN) on AC-induced connexin 43 (Cx43) lateralization. A–C: Representative immunohistochemical staining of left atrium (LA) using specific Cx43 primary antibodies in control group (A), AAC-vehicle (VEH) group (B), and AAC-CAN group (C). D: Quantitative analysis of Cx43 lateralization. White ellipses in B indicate lateralization of Cx43. Data are mean ± standard error of the mean (SEM). $n=8$ in each group. $^{*}p < 0.05$, $^{**}p < 0.01$.

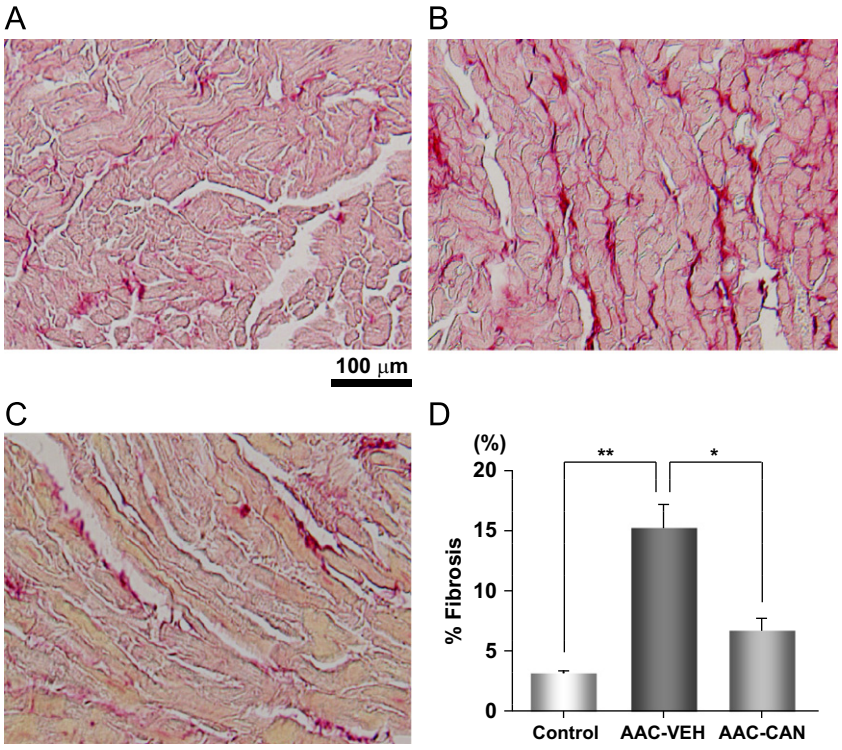

Fig. 11. Effects of candesartan (CAN) on fibrosis induced by abdominal aortic constriction (AAC). A–C: Representative left atrium (LA) free wall sections by Sirius red staining in control group (A), AAC-vehicle (VEH) group (B) and AAC-CAN group (C). D: Quantitative analysis of LA fibrosis. Data are mean ± standard error of the mean (SEM). $n=8$ in each group. $^{*}p<0.05$, $^{**}p<0.01$.

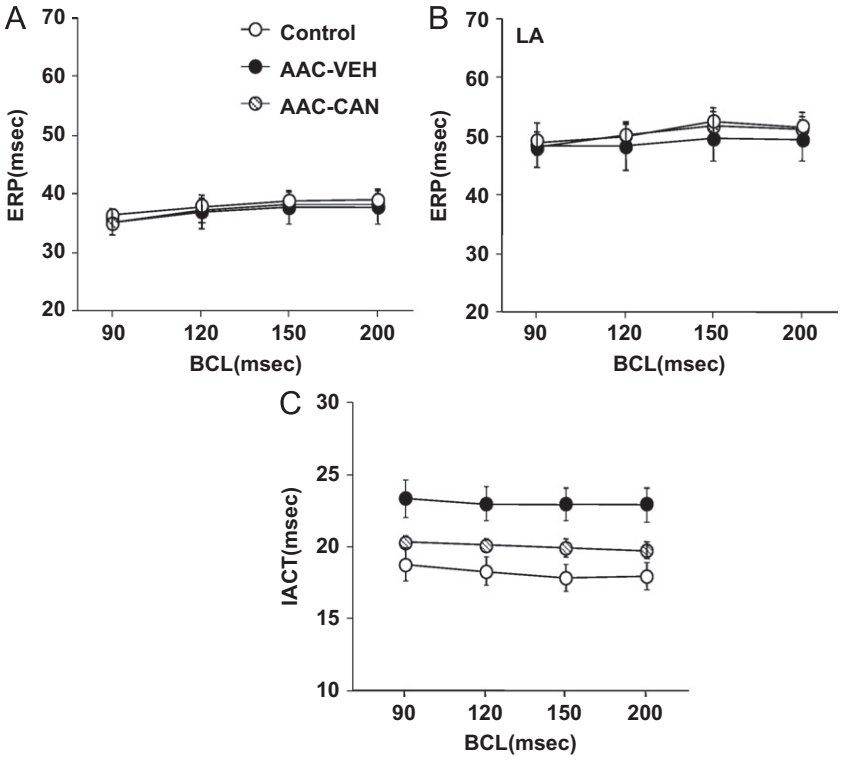

Fig. 12. Electrophysiological characteristics. A: Effective refractory period (ERP) of the right atrium (RA). B: ERP of the left atrium (LA). C: Interatrial conduction time (IACT). ERP and IACT were measured at basic cycle lengths (BCLs) of 200, 150, 120, and 90 ms. Data are mean ± standard error of the mean (SEM). $n=8$ in each group. $^{**}p<0.01$ vs. control group.

candesartan (Fig. 10). Fig. 11 shows representative histological sections of left atrium. Compared with the control group (Fig. 11A), extensive and heterogeneous interstitial fibrosis was observed in the AAC-treated group (Fig. 11B). The interstitial fibrosis induced by AAC was attenuated by candesartan (Fig. 11C). Fig. 12A and B depict the atrial ERP at 4 basic cycle lengths (BCL)

of 200, 150, 120, and 90 ms. In both the right and left atrium no significant differences in ERP were observed among the 3 groups. Compared with the control group, the AAC-treated group showed prolonged interatrial conduction time in all BCL tested (Fig. 12C). This prolongation was inhibited by candesartan treatment. In an electrophysiological study using isolated perfused heart, AF was not induced in hearts from the control group. In the AAC-treated group, S3 extrastimuli induced AF in 9 of 10 rats (90%). In the AAC-candesartan group, AF induction was observed in only 1 of 10 rats (10%). Treatment with candesartan, therefore, resulted in a significant reduction in the incidence of AF in the AAC-treated hearts (data not shown).

Redistribution of Cx43 was shown in rats after 24-h of atrial tachypacing-induced AF [44,45]. The extent of connexin distribution was shown to correlate with AF duration in goat atrial tachypacing AF model [46]. The effects of pressure overload on the cardiac gap junction have been poorly investigated. Emdad et al. [47] reported that rats developed left ventricular hypertrophy, in which Cx43 lateralization was evident at 12 weeks after abdominal AC. Remodeling of the gap junction in atria by AAC has not been reported. Rucker-Martin et al. [48] studied Cx43 distribution in patients with valvular diseases and dilated atria. In patients characterized by an elevated pulmonary arterial pressure, atrial Cx43 was dephosphorylated and lateralized. Cx43 lateralization extended to a similar degree in patients with sinus rhythm and AF [48]. Together with our observation that AAC alone caused Cx43 lateralization, we hypothesize that hemodynamic pressure overload, without tachypacing, is sufficient to provide conditions that induce Cx43 remodeling.

Candesartan prevented Cx43 lateralization independent of blood pressure-lowering and anti-hypertrophic effects. Possible linkage between AT1 receptor activation and Cx43 expression has been shown to occur at a cellular level [49]. By using cultured neonatal rat cardiomyocytes, incubation with AII increased cellular Cx43 expression and its phosphorylation in a concentration-dependent manner, which was completely inhibited by losartan [49]. Based on signaling pathway investigations, the authors concluded that Erk and p38 signaling pathways were involved in the regulation of Cx43 expression by AII [49]. In addition, cyclic stretch of cultured neonatal rat cardiomyocytes was shown to result in increased Cx43 expression and AII release into the culture media, which was attenuated by losartan [50]. Using neonatal rat-cultured cardiomyocytes rapid electrical stimulation of contraction resulted in significant upregulation of Cx43 and increased AII content, in association with an increase in conduction velocity by extracellular potential mapping using a multiple electrode array system [51]. These effects induced by rapid electrical stimulation were prevented by losartan and specific inhibitors of Erk and p38 signaling. Therefore, the authors concluded that a short-term rapid electrical stimulation causes Cx43 upregulation and a concomitant increase in conduction velocity, mainly through activation of Erk and p38 via an autocrine action of AII [51]. These cellular-level experiments have demonstrated that stimuli, including AII, mechanical stretch, and rapid electrical pacing, invariably result in Cx43 upregulation, possibly leading to an increase in the conduction. It is difficult to applying these in vitro findings to experimental in vivo findings, including that in our study. In the present study, slowing of interatrial conduction occurred in association with Cx43 lateralization. Lateralized connexins have been shown to be nonfunctional, probably leading to slow conduction [48]. In addition to direct pressure overload by AAC other mechanisms may contribute to Cx43 lateralization. Notable interstitial fibrosis may be involved in the processes for Cx43 lateralization. It has been suggested that interstitial fibrosis may alter the cyoskeletal network and adhesion proteins that contribute to the normal organization of connexins [52]. Our observations could not clarify whether Cx43 lateralization or fibrosis has a more dominant role for slowing of interatrial conduction. Further studies are required to clarify the specific role of gap junction remodeling in relation to fibrosis in the interatrial conduction disturbance induced by pressure overload. Nevertheless, AT1 receptor blockers, including candesartan, may be expected to prevent atrial fibrosis, gap junction remodeling and enhanced AF vulnerability, especially under the pathogenic condition where the renin-angiotensin system is activated.

4. Conclusions

In a clinical setting, the results observed with upstream therapies appear to be less encouraging. For instance, in GISSI-AF trial, [53] treatment with valsartan was not associated with a reduction in the incidence of recurrent AF. This is in contrast to the results obtained in animal experiments, including our study, which compellingly shows the protective effect of angiotensin-converting enzyme inhibitors, ARBs, statins, and *n*-3 polyunsaturated fatty acids against electrical and structural atrial remodeling in association with AF [7,8]. One possible explanation is that most of the clinical studies investigated the recurrence of AF, the secondary prevention. In patients who have previously developed AF, atrial structural remodeling may be unexpectedly progressed, and many mechanisms may be involved. In such conditions, the above agents will be less likely to prevent the recurrence of AF as other pathways will still be at play. However, it could be possible that upstream therapies can reduce new-onset AF. In addition, upstream therapies may impact mortality and cardiovascular events in this population. Therefore, sophisticated experimental studies, possessing significant clinical relevance, should be challengingly continued.

Conflict of interest

None.

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