Contents lists available at ScienceDirect

Indian Pacing and Electrophysiology Journal

Serum level of transforming growth factor beta 1 is associated with left atrial voltage in patients with chronic atrial fibrillation

Shilu Zhao, Mingfang Li, Weizhu Ju, Lingyun Gu, Fengxiang Zhang, Hongwu Chen, Kai Gu, Bing Yang, Minglong Chen[*]

Cardiology Division, The First Affiliated Hospital of Nanjing Medical University, Nanjing, PR China

A B S T R A C T

Background: Atrial tissue fibrosis can cause electrical or structural remodeling in patients with atrial fibrillation. Transforming growth factor beta 1(TGF-β1) signaling acts as a central role in fibroblast activation. In this report, we aimed to investigate the relationship between serum level of TGF-β1 and mean left atrial voltage in patients with chronic atrial fibrillation (CAF).
Methods: A total of 16 consecutive adult patients with CAF who underwent catheter ablation were enrolled. Blood samples for measurement of TGF-β1 were collected from periphery veins and coronary sinus before pulmonary vein isolation. The measurement was performed with a commercially available ELISA kit. Cardiac indices were measured using echocardiography. The left atrial electroanatomic mapping was performed after pulmonary vein isolation.
Results: Serum level of TGF-β1 in peripheral blood was higher than that in coronary sinus (p < 0.001). TGF-β1 serum level in coronary sinus negatively correlated with mean left atrial voltage (r = -0.650, p = 0.012), While periphery TGF-β1 level tended to be negatively correlated with mean left atrial voltage(r = -0.492, p = 0.053). Patients who treated with angiotensin II receptor antagonists had lower coronary sinus TGF-β1 serum level than those who did not treated with angiotensin II receptor antagonists (p = 0.046).
Conclusion: Level of TGF-β1 in peripheral serum is higher than that in coronary sinus, and serum level of TGF-β1 in coronary sinus is negatively associated with mean left atrial voltage in patients with CAF, angiotensin II receptor antagonists could affect TGF-β1 serum level.

1. Introduction

Atrial fibrillation (AF) is the most common clinical arrhythmia that occurs in individuals with a variety of cardiovascular diseases or without any other evidence of systemic diseases [1]. It affects 5% of the population older than 65 years and the prevalence increases as the population age arises [2]. Despite of more and more research on the mechanisms of AF, the exact cause and pathogenesis of AF remains unclear [3–5]. Two principal forms of remodeling have been described in animal models of AF: electrical remodeling, which affects cellular electrical properties, and structural remodeling, which alters atrial tissue architecture [6]. Structural

remodeling can be caused by interstitial fibrosis.

Atrial fibrosis, a detrimental process that causes imbalance in extracellular matrix deposition and degradation, has been implicated as a substrate for AF. However, the precise mechanisms of structural remodeling and the relationship between atrial fibrosis and atrial fibrillation were largely unknown. Recent experimental and clinical studies have provided valuable insights on the mechanisms of atrial fibrosis at molecular and cellular level. A variety of signaling systems, particularly involving angiotensin II and transforming growth factor-β1 (TGF-β1), seem to be centrally involved in the promotion of fibrosis [7]. Angiotensin II promotes aldosterone secretion to increases mRNA levels of TGF-β1, and converts TGF-β1 into its active form. Aminopeptidase A converts angiotensin II into angiotensin III, which also increases TGF-βl expression [8,9]. TGF-β1 is a subtype of TGF-β. TGF-β signaling was implicated in the pathogenesis of fibrotic diseases by regulating the expression of other proteins involved in executing the fibrotic cascade [10]. Using

* Corresponding author. Division of Cardiology, The First Affiliated Hospital of NanjingMedical University, No. 300, Guangzhou Road, 210029 Nanjing, PR China.
E-mail address: chenminglong@njmu.edu.cn (M. Chen).

Peer review under responsibility of Indian Heart Rhythm Society.

human atrial myocardial tissue, Kupfahl et al. noted that angiotensin II might up-regulate the expression of TGF-β1, and TGF-β1 signaling affects collagen production [11]. Extracellular matrix changes can separate cardiomyocyte bundles, which can diminish electrical coupling and slow electrical conduction [12,13]. It is believed that localized atrial fibrosis may complex atrial electrograms and decrease voltage, while diffuse and profound fibrosis can make the local tissue scarred. These fibrosis related with electrophysiologic changes can be represented by electroanatomic bipolar voltage mapping [14—19].

Overall, TGF-β is a critical regulator of extracellular matrix production, and extracellular matrix changes could be reflected by electroanatomical alternations. Therefore, it is potential for further comprehensive research on whether electroanatomical alternations have correlation with serum TGF-β1 level. The aim of this study is to investigate the relationship between mean left atrial voltage and serum level of TGF-β1 in patients with chronic atrial fibrillation (CAF).

2. Methods

2.1. Participants

We retrospectively analyzed a total of 16 consecutive adult patients with drug refractory CAF who underwent catheter ablation for AF using 3D mapping system (NavX, St. Jude Medical Inc., St. Paul, MN, USA) were enrolled at a single university medical center from June 2012 to May 2013. The definition of CAF was based on the Holter monitoring electrocardiogram, requiring the characters that there was the rhythm of AF instead of sinus rhythm by every time checking. Patients were excluded if they were likely to be elevated serum TGF-β1 level: patients with history of myocardial infarction or elevated level of troponin, angina pectoris, thyroid disease, vascular heart disease, hypertrophic cardiomyopathy, chronic kidney disease, chronic lung disease, chronic liver disease, autoimmune disease, any acute rheumatologic or infectious disease, any trauma or surgery. Congenital heart disease was also excluded from this study. All patients provided written informed consent to the study protocol, which was approved by the Human Research Ethics Committee of the first affiliated Hospital of Nanjing Medical University.

2.2. Fibrosis factor of TGF-β1

After local anesthesia, patient underwent placement of intravenous sheaths in three periphery and subclavian vein. A coronary sinus catheter was placed for electrophysiology study through subclavian vein. When the catheter and sheaths had been placed but before interatrial septum piercing, blood was withdrawn from both the coronary sinus and one of the periphery veins. The first 10 cc of blood was discarded, and the second 10 cc were obtained for TGF-β1 measurement. All blood samples were centrifuged at 2000 g for 10 min, serums were extracted and stored at −80 °C. Blood withdrawn from coronary sinus was confirmed by three steps: intracardiac catheter consistent with coronary sinus placement, the fluoroscopic image of the catheter in standard right and left anterior oblique, and the gross darker appearance of blood than withdrawn from the periphery veins. Measurement of serum TGF-β1 level was performed with a commercially available immunoassay/ELISA.

2.3. Electroanatomic mapping

All AF patients underwent transesophageal echocardiography on the day of the study to exclude left atrium (LA) thrombus. After catheter placement, three-dimensional (3D) geometries of LA and pulmonary veins were created separately using the A-Focus catheter coupled with EnSite-NavX. For all patients, high density mapping were achieved after circumferential pulmonary vein isolation and electric cardioversion were required to restore sinus rhythm (Fig. 1). Only mapping points outside of the pulmonary veins were used for analysis [20]. Local voltage was defined as the amplitude from the peak-positive to the peak-negative deflection of the local bipolar electrogram. The average bipolar mapping sites in the LA were 237 ± 72 points for each patient respectively, and the mean peak-to-peak voltage throughout the entire LA was calculated.

2.4. Statistical analysis

All the data were described by mean ± standard deviation. The correlation between various parameters and LA voltage properties were evaluated with Pearson's correlation coefficients. For comparison between groups, the data were analyzed by independent t-test. As the distribution of variables were highly skewed, TGF-β1 level and each segments of the voltage value were log-transformed to normalize their distribution before statistical analysis. All analyses were performed by SPSS software (version 13.0, SPSS Inc., Chicago, Illinois). The P-value reported was two-sided and the value of less than 0.05 was considered statistically significant.
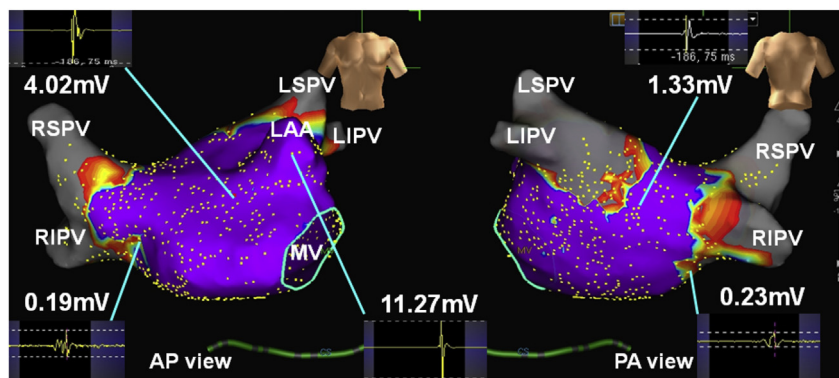
Fig. 1. The example of high density mapping achieved after circumferential pulmonary vein isolation and electric cardioversion to restore sinus rhythm.
LAA = left atrial appendage; LIPV = left inferior pulmonary vein; LSPV = left superior pulmonary vein; RIPV = right inferior pulmonary vein; RSPV = right superior pulmonary vein; MV = mitral valve.

3. Results

A total of 16 patients were enrolled in this study. Baseline clinical characteristics are listed in Table 1. This study had more male patients than female. Mean age of the study subjects was 57 years. Four patients had hypertension. Mean duration of CAF was 36 months before the radiofrequency ablation. Most of the patients took several kinds of drugs for treatment except two. Thirteen (81.25%) of the patients used β-blocker, five (31.25%) used angiotensin II receptor antagonists.

Fourteen of the patients had coronary sinus blood drawn for further detection of TGF-β1. Both periphery and coronary sinus TGF-β1 level had no correlation with age, duration of CAF, LVEF and LAD in the limited sample size (Table 2).

TGF-β1 serum level in coronary sinus was negatively correlated with mean left atrial voltage ($r = -0.650$, $p = 0.012$), while periphery TGF-β1 level tended to be negatively correlated with mean left atrial voltage($r = -0.492$, $p = 0.053$) (Fig. 2). It was found that patients who treated with angiotensin II receptor antagonists had lower level of TGF-β1 in coronary sinus ($p = 0.046$).

Table 1
Baseline characteristics of enrolled patients.

Characteristics (N = 16)	Estimates
Age, year old	57 ± 11
Gender, n (%)	
Male	12 (75)
Female	4 (25)
Cardiovascular risk factors, n (%)	
Hypertension	4 (25)
Diabetes	0 (0)
Cardiac family history	2 (12.5)
Smoking current	8 (50)
Hypercholesterinemia	1 (6.25)
Duration of AF before radiofrequency ablation, months	
Mean ± SD	36 ± 36
Median(interquartile range)	24 (1−120)
Drug treatment	
Angiotensin II receptor antagonists	5 (31.25)
β-blocker	13 (81.25)
Ca^{2+} channel blocker	1 (6.25)
Diuretics	1 (6.25)
Cardiac indices	
LV EF (%)	62 ± 6
LAD (mm)	43 ± 4
LVSD (mm)	32 ± 7
LVDD (mm)	49 ± 6
IVS (mm)	10 ± 1
LVPW (mm)	10 ± 1
TGF-β1 level (ng/L)	
In periphery veins	1191.1 ± 724.0
In coronary sinus	676.8 ± 526.2
Left atrial mean voltage (mV)	2.32 ± 0.7

LVEF = left ventricular ejection fraction; LAD = left atrial diameter; LVSD = left ventricular end systolic diameter; LVDD = left ventricular end diastolic diameter; IVS = interventricular septum; LVPW = left ventricular posterior wall; TGF-β1 = transforming growth factor-β1.

Table 2
Correlation between TGF-β1 and clinical characteristics.

	Periphery TGF-β1		Coronary sinus TGF-β1	
	r	p value	r	p value
Age	0.080	0.768	0.209	0.472
Duration of symptomatic AF	0.021	0.937	−0.075	0.800
LV ejection fraction	0.035	0.899	0.129	0.660
Left atrial diameter	0.288	0.279	0.010	0.974
Left atrial mean voltage	−0.492	0.053	−0.650	0.012
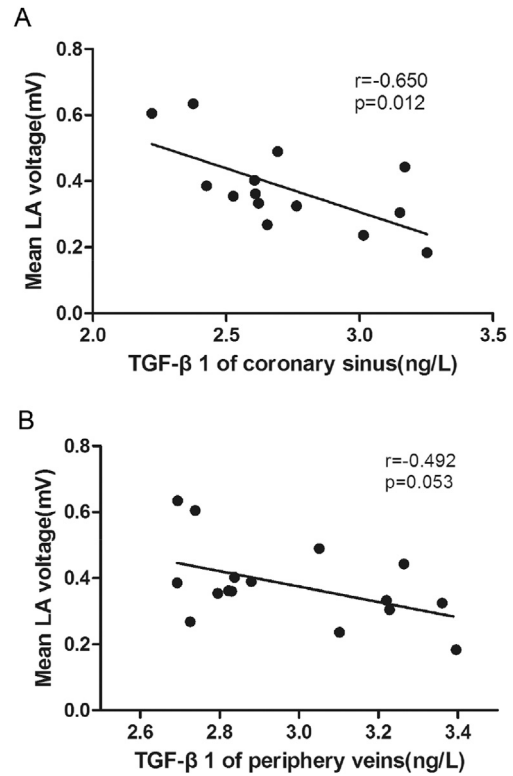
Fig. 2. **A:** Relationship between TGF-β1 serum level in coronary sinus and mean left atrial voltage. TGF-β1 serum level in coronary sinus was negatively correlated with mean left atrial voltage ($r = -0.650$, $p = 0.012$). **B:** Relationship between periphery TGF-β1 level and mean left atrial voltage. Periphery TGF-β1 level tended to be negatively correlated with mean left atrial voltage ($r = -0.492$, $p = 0.053$).

4. Discussion

4.1. Major findings

The major findings of the present study are as followings: (1) peripheral TGF-β1 level were higher than coronary sinus obviously; (2) serum level of TGF-β1 in coronary sinus has negative correlation with left atrial mean voltage. I.e. TGF-β1 in coronary sinus has positive correlation with atrial fibrosis. Serum level of TGF-β1 in periphery blood did not has correlation with left atrial mean voltage significantly, but it indicated that it trends to show negative correlation between them in our limited sample. (3) TGF-β1 level has correlation with using of angiotensin II receptor antagonists. Those patients who used angiotensin II receptor antagonists had lower TGF-β1 serum level in coronary sinus than those without treatment of angiotensin II receptor antagonists.

4.2. Earlier studies

Studies have shown that patients with AF exist with atrial fibrosis, which is the common feature of AF [21]. Histological study had proved that increased myocardial collagen deposition was found in patients with AF compared to those with sinus rhythm [22]. Substantial evidence supports that TGF-β1 acts as a central role in fibroblast activation. Wounds treated with anti-TGF-β1 antibodies were shown to reduce extracellular matrix synthesis and scarring [23]. TGF-β1 promotes extracellular matrix deposition by inducing tissue inhibitors of matrix metalloproteinase gene expression and suppressing matrix metalloproteinase gene expression [24]. Normal myocardial cells have a high degree of

electrical coupling. Normal conduction between myocardial cells depends on the number of myocytes, the electric coupling and conduction characteristics. When atrial fibrosis occurs, there is not only a loss of atrial myocytes because of cell necrosis or apoptosis, but also an increase in interstitial space between cardiomyocytes due to the accumulation of fibrillar collagen [25,26]. Therefore, atrial fibrosis can diminish electrical coupling and slow electrical conduction, which may be reflected by low atrial voltage. In an animal model, the lowest bipolar voltage was detected in the fibrosed posterior wall presumably induced by localized atrial dilation [27]. TGF-β1 does not act independently on cardiac remodeling and fibrosis. Recent studies [28,29] indicated that TGF-β1 functioned as a mediator of angiotensin II. Angiotensin receptor inhibitors such as losartan appear to increase cell-to-cell communication and be effective in reducing cardiac fibrosis in various models of animals and human.

4.3. Interpretation of the present results

In the present study, peripheral TGF-β1 level were higher than coronary sinus obviously, we consider that TGF-β1 act as an mediator of angiotensin, which has impact on multiple systems such as heart, brain, and kidney, so peripheral TGF-β1 level reflects the results of multiple systemic fibrosis while coronary sinus level reflects the part of ventricular venous blood. And the results could be considered as indirect evidence that TGF-β1 act as a part of an integrated signaling network which promotes cardiac fibrosis and structure remodeling result in electrical remodeling of myocardial cells. In the study, we can conclude that angiotensin II receptor antagonists reduce cardiac fibrosis partly by suppressing TGF-β1 expression.

4.4. Limitation

To the best of our knowledge, this is the first attempt to study the association between the left atrial voltage properties and serum level of TGF-β1, especially in coronary sinus. However, there are several limitations in the study. First, the sample size of the study was small and even though the P value for correlation is significant between serum level of TGF-β1 in coronary sinus and LA voltage, the r or r^2 value is not very large, so our results need to be confirmed with further studies with large sample size. Second, patients enrolled in this study only underwent left atrial electro-anatomic mapping. Right atrial and ventricular mapping were not performed. Besides, although the points within the pulmonary veins were excluded, the voltage of the area surrounding the pulmonary veins may be reduced, which may also affect the results. In addition, serum level of TGF-β1 should be directly compared to cardiac tissue fibrosis. However, that was beyond the present study.

5. Conclusion

In this single center cohort study, it was demonstrated that the level of TGF-β1 was higher in peripheral serum than that in coronary sinus, and serum level of TGF-β1 in coronary sinus was negatively correlated with left atrial mean voltage in patients with CAF. Patients who treated with angiotensin II receptor antagonists had lower coronary sinus TGF-β1 serum level than those who did not treated with angiotensin II receptor antagonists. These results could be considered as an indirect evidence that TGF-β1 act as a part of an integrated signaling network which promotes cardiac fibrosis and angiotensin receptor inhibitors could be used for delay the progression of atrial fibrillation.

Conflict of interest

All authors declare no conflict of interest related to this study.

References

[1] Nattel S. New ideas about atrial fibrillation 50 years on. Nature 2002 Jan 10;415(6868):219–26.

[2] Tan AY, Zimetbaum P. Atrial fibrillation and atrial fibrosis. J Cardiovasc Pharmacol 2011 Jun;57(6):625–9.

[3] Calò L, Martino A, Sciarra L, Ciccaglioni A, De Ruvo E, De Luca L, et al. Upstream effect for atrial fibrillation: still a dilemma? Pacing Clin Electrophysiol 2011 Jan;34(1):111–28.

[4] Zhang P, Wang W, Wang X, Wang X, Song Y, Han Y, et al. Protein analysis of atrial fibrosis via label-free proteomics in chronic atrial fibrillation patients with mitral valve disease. PLoS One 2013 Apr 4;8(4):e60210.

[5] Huntgeburth M, Tiemann K, Shahverdyan R, Schlüter KD, Schreckenberg R, Gross ML, et al. S Transforming growth factor β1 oppositely regulates the hypertrophic and contractile response to β-adrenergic stimulation in the heart. PLoS One 2011;6(11):e26628.

[6] Nattel S, Shiroshita-Takeshita A, Cardin S, Pelletier P. Mechanisms of atrial remodeling and clinical relevance. Curr Opin Cardiol 2005 Jan;20(1):21–5.

[7] Goudis CA, Kallergis EM, Vardas PE. Extracellular matrix alterations in the atria: insights into the mechanisms and perpetuation of atrial fibrillation. Europace 2012 May;14(5):623–30.

[8] Naito T, Masaki T, Nikolic-Paterson DJ, Tanji C, Yorioka N, Kohno N. Angiotensin II induces thrombospondin-1 production in human mesangial cells via p38 MAPK and JNK: a mechanism for activation of latent TGF-beta1. Am J Physiol Ren Physiol 2004 Feb;286(2):F278–87.

[9] Wenzel S, Taimor G, Piper HM, Schlüter KD. Redox-sensitive intermediates mediate angiotensin II-induced p38 MAP kinase activation, AP-1 binding activity, and TGF-beta expression in adult ventricular cardiomyocytes. FASEB J 2001 Oct;15(12):2291–3.

[10] Sales VL, Engelmayr Jr GC, Mettler BA, Johnson Jr JA, Sacks MS, Mayer Jr JE. Transforming growth factor-beta1 modulates extracellular matrix production, proliferation, and apoptosis of endothelial progenitor cells in tissue-engineering scaffolds. Circulation 2006 Jul 4;114(1 Suppl):I193–9.

[11] Kupfahl C, Pink D, Friedrich K, Zurbrügg HR, Neuss M, Warnecke C, et al. Angiotensin II directly increases transforming growth factor beta1 and osteopontin and indirectly affects collagen mRNA expression in the human heart. Cardiovasc Res 2000 Jun;46(3):463–75.

[12] Kawara T, Derksen R, de Groot JR, Coronel R, Tasseron S, Linnenbank AC, et al. Activation delay after premature stimulation in chronically diseased human myocardium relates to the architecture of interstitial fibrosis. Circulation 2001 Dec 18;104(25):3069–75.

[13] Spach MS, Dolber PC. Relating extracellular potentials and their derivatives to anisotropic propagation at a microscopic level in human cardiac muscle. Evidence for electrical uncoupling of side-to-side fiber connections with increasing age. Circ Res 1986 Mar;58(3):356–71.

[14] Kistler PM, Sanders P, Fynn SP, Stevenson IH, Spence SJ, Vohra JK, et al. Electrophysiologic and electroanatomic changes in the human atrium associated with age. J Am Coll Cardiol 2004 Jul 7;44(1):109–16.

[15] Verma A, Wazni OM, Marrouche NF, Martin DO, Kilicaslan F, Minor S, et al. Pre-existent left atrial scarring in patients undergoing pulmonary vein antrum isolation: an independent predictor of procedural failure. J Am Coll Cardiol 2005 Jan 18;45(2):285–92.

[16] Chang SL, Tai CT, Lin YJ, Wongcharoen W, Lo LW, Tuan TC, et al. Biatrial substrate properties in patients with atrial fibrillation. J Cardiovasc Electrophysiol 2007 Nov;18(11):1134–9.

[17] Stiles MK, John B, Wong CX, Kuklik P, Brooks AG, Lau DH, et al. Paroxysmal lone atrial fibrillation is associated with an abnormal atrial substrate: characterizing the "second factor". J Am Coll Cardiol 2009 Apr 7;53(14):1182–91.

[18] Teh AW, Kistler PM, Lee G, Medi C, Heck PM, Spence SJ, et al. Electroanatomic remodeling of the left atrium in paroxysmal and persistent atrial fibrillation patients without structural heart disease. J Cardiovasc Electrophysiol 2012 Mar;23(3):232–8.

[19] Marcus GM, Yang Y, Varosy PD, Ordovas K, Tseng ZH, Badhwar N, et al.

Regional left atrial voltage in patients with atrial fibrillation. Heart Rhythm 2007 Feb;4(2):138—44.

[20] Lin Y, Yang B, Garcia FC, Ju W, Zhang F, Chen H, et al. Comparison of left atrial electrophysiologic abnormalities during sinus rhythm in patients with different type of atrial fibrillation. J Interv Card Electrophysiol 2014 Jan;39(1): 57—67.

[21] Kostin S, Klein G, Szalay Z, Hein S, Bauer EP, Schaper J. Structural correlate of atrial fibrillation in human patients. Cardiovasc Res 2002 May;54(2):361—79.

[22] Frustaci A, Chimenti C, Bellocci F, Morgante E, Russo MA, Maseri A. Histological substrate of atrial biopsies in patients with lone atrial fibrillation. Circulation 1997 Aug 19;96(4):1180—4.

[23] Shah M, Foreman DM, Ferguson MW. Neutralising antibody to TGF-beta 1,2 reduces cutaneous scarring in adult rodents. J Cell Sci 1994 May;107(Pt 5): 1137—57.

[24] Leask A. Targeting the TGFbeta, endothelin-1 and CCN2 axis to combat fibrosis in scleroderma. Cell Signal 2008 Aug;20(8):1409—14.

[25] Assayag P, Carré F, Chevalier B, Delcayre C, Mansier P, Swynghedauw B. Compensated cardiac hypertrophy: arrhythmogenicity and the new myocardial phenotype. I. Fibrosis. Cardiovasc Res 1997 Jun;34(3):439—44.

[26] Silver MA, Pick R, Brilla CG, Jalil JE, Janicki JS, Weber KT. Reactive and reparative fibrillar collagen remodelling in the hypertrophied rat left ventricle: two experimental models of myocardial fibrosis. Cardiovasc Res 1990 Sep;24(9): 741—7.

[27] Huang JL, Tai CT, Lin YJ, Ting CT, Chen YT, Chang MS, et al. The mechanisms of an increased dominant frequency in the left atrial posterior wall during atrial fibrillation in acute atrial dilatation. J Cardiovasc Electrophysiol 2006 Feb;17(2):178—88.

[28] De Mello WC, Specht P. Chronic blockade of angiotensin II AT1-receptors increased cell-to-cell communication, reduced fibrosis and improved impulse propagation in the failing heart. J Renin Angiotensin Aldosterone Syst 2006 Dec;7(4):201—5.

[29] Shibasaki Y, Nishiue T, Masaki H, Tamura K, Matsumoto N, Mori Y, et al. Impact of the angiotensin II receptor antagonist, losartan, on myocardial fibrosis in patients with end-stage renal disease: assessment by ultrasonic integrated backscatter and biochemical markers. Hypertens Res 2005 Oct;28(10):787—95.