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TGF-β1 signal pathway in the regulation of inflammation in patients with atrial fibrillation

Ye Erbo lati· Ali Mira[1], Muhuyati[1*], Wu-Hong Lu[2], Peng-Yi He[1], Zhi-Qiang Liu[1], Yu-Chun Yang[1]

[1]*Comprehensive Cardiology Department, First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830011, China*

2Cardiac Institute, First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830011, China

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ABSTRACT

Objective: To observe the expression changes of inflammatory markers TGF-β1, Smad3 and IL-6 in patients with atrial fibrillation (AF), and to explore the significance of TGF-β1 signaling pathway in the structural remodeling of AF. **Methods:** The expression of TGF-β1, Smad3 and IL-6 in 50 cases with AF and 30 normal cases were detected by RT-PCR and ELISA. **Results:** The TGF-β1, Smad3 and IL-6 mRNA and protein expression levels in patients with AF were significantly higher than that in the control group ($P<0.05$), but there was no significantly different between the paroxysmal AF group and the persistent AF group ($P>0.05$). The TGF-β1mRNA expression in the ≥50 years subgroup was significantly higher than that in the <50 years subgroups, and it was higher in the NYHA Ⅲ subgroup than in the Ⅰ/Ⅱ grade subgroup. It was also higher in the left ventricular ejection fraction (LVEF) <50% subgroup than in LVEF≥50% group, and it was significantly higher in the AF time ≥36 months subgroup than that in <36 months subgroup ($P<0.05$). The Smad3 and IL-6 expressions in the in the LVEF <50% subgroup were both high that than that in LVEF≥50% group, and higher in the AF time ≥36 months subgroup than that in <36 months subgroup ($P<0.05$). There were a positive correlation between TGF-β1, Smad3 and IL-6 ($r=0.687$, $r=0.547$). There were also a positive correlation between Smad3 and IL-6 mRNA ($r=0.823$). **Conclusions:** AF is associated with inflammation, and the inflammation is also involved in the fibrillation and sustain of AF. The TGF-β1 signal pathway may be involved in the process of atrial structural remodeling in patients with AF, and iss related with the occurrence and maintenance of AF.

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinical, and the incidence rates are increased with age gradually. It can reduce the primary pump function of the atrium, increase ventricular burden, which can easily lead to heart failure, and finally increase the mortality. Currently the pathogenesis remains unclear, but a growing number of studies suggest that the occurrence and development of AF are closely related to inflammation. In this study, in order to explore the relationship between the TGF-β1, Smad3 and IL-6 expressions and the AF, TGF-β1, Smad3 and IL-6 expression levels in patients with AF in plasma were detected by RT-PCR and ELISA.

2. Materials and methods

2.1. Clinical data

All 50 subjects were patients with AF admitted from June

*Corresponding author: Muhuyati, Chief Physician, Professor and Doctoral Supervisor, Comprehensive Cardiology Department, First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang 830011, China.
Fax: 0991-4366334
Tel: 13619926334
Email: xingas008@126.com
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2008 to October 2010, including 30 males and 20 females, aged from 36 to 68 years old, with the average age as (49.1±10.6) years old. Another 30 healthy persons were selected during the same period as control group, including 16 males and 14 females, aged from 37 to 62 years old, with the average age as (50.1±10.1) years old. There was no significant difference in age (t=0.587, P=0.691) or gender (t=0.341, P=0.559) between two groups. In control group, there were 22 cases at Ⅰ/Ⅱ grade, and 8 cases at Ⅲ grade; while in AF group, there were 38 cases at Ⅰ/Ⅱ grade, and 12 cases at Ⅲ grade, with no significant difference between two groups (t=0.071, P=0.790). Patients with left ventricular ejection fraction=45% due to any other structural heart disease or cardiovascular disease were excluded, and patients with infectious or non-infectious inflammatory disease, acute coronary syndrome, severe liver and kidney dysfunction, cancer, immune system diseases, tissue damage within a month and vascular events, surgery and stroke within six months were all excluded. According to the criteria, the subjects were divided into paroxysmal AF group (13 cases) and persistent AF group (37 cases) according to whether they could convert to sinus rhythm spontaneously. They had physical examination, routine laboratory tests, electrocardiogram (conventional, 24 h dynamic), chest X-ray, thyroid function tests, and echocardiography. Transthoracic and/or transesophageal echocardiography examination were carried out to exclude potential patients with structural heart disease during the same time. Patients did not receive any angiotensin-converting enzyme inhibitors or drugs in angiotensin Ⅱ receptor blocker class within six months before surgery. There was no statistically significant difference in age, gender, body weight, and all patients have signed an informed consent.

2.2. Reagents

TGF-β1, Smad3 and IL-6 ELISA kits were all purchased from Shenzhen Jingmei Company. Total RNA extraction kits were purchased from Shanghai Sangon Company. TGF-β1 (185 bp) upstream primers: 5′-CACCCAGCCCACGAATCATCTCC-3′, downstream: 5′-GGGCCCCCTTCTCTCTACCTG-3′; Smad3 (236 bp) upstream primers: 5′-GGGGATACCAGCAGCAAGAGAG-3′, downstream: 5′-CGGCTGATGCTCCTTAAACTGT-3′; IL-6 (182 bp) upstream primers: 5′-GGAGACGCCTTGAAGTAACTGC-3′, downstream: 5′-GAGTTTCCTCTGACTCCATCGCAG -3′; Internal reference sequence GADPH (142 bp) upstream: 5′-GCACCGTCAAGGCTGAGAAC-3′, downstream: 5′-ATGGTGGTGAAGACGCCAGT-3′, which were all synthesized by Shanghai Sangon Biological Engineering Co., Ltd.

2.3. Experimental methods

5 mL fasting quiet state peripheral cubital vein blood were collected, and then placed in EDTA anticoagulant tube for 0.5 h, centrifuged at 3 000 r/min for 5 min. Upper layer serum was collected and stored at -80 ℃, the same batch was detected in 2 months. TGF-β1, Smad3 and IL-6 protein were detected by enzyme-linked immunosorbent assay in accordance with the kit instructions. TGF-β1, Smad3 and IL-6 mRNA was detected by detecting total RNA, the extraction kits were purchased from Shanghai Sangon Company.

2.4. Result determination

Positive band at 85 bp, 236 bp, 182 bp and 142 bp were identified as TGF-β1, Smad3, IL-6 and GADPH mRNA expression respectively; GADPH was considered as an internal reference, the grayscale integral value of each stripe were recorded by computer, statistical analysis was performed by sample integral value/internal reference ratio.

2.5. Statistical analysis

All data were analyzed by SPSS 13.0 statistics software, t-test, variance analysis, *Chi*-square test and the Spearman rank correlation analysis were applied. P<0.05 was regarded as statistical significant difference.

3. Results

3.1. General information of patients in two groups

In 30 patients of the control group, left ventricular dysfunction (LVD), left anterior descending artery (LAD), right anterior descending artery (RAD), left ventricular ejection fraction (LVEF), resting heart rate, systolic and diastolic blood pressure were (47.9±4.5) mm, (55.7±8.5) mm, (38.4±4.9) mm, (63.9±4.5)%, respectively. Resting heart rate (78.5±18.6) beats/min, systolic blood pressure (127.6±29.0) mmHg and diastolic blood pressure (85.6±15.2) mmHg. In 50 patients of the AF group , LVD, LAD, RAD, LVEF, resting heart rate, systolic and diastolic blood pressure were (50.3±4.3) mm, (58.3±6.6) mm, (35.9±6.2) mm, (58.4±7.0)%, respectively.Resting heart rate (87.8±15.2) beats/min,systolic blood pressure (123.8±38.5) mmHg and diastolic blood pressure (82.8±14.0) mmHg. There were no significant difference in LVD (t=0.857, P=0.387), LAD (t=0.583, P=0.724), RAD (t=0.526, P=0.780), LVEF (t=1.534, P=0.126), resting

heart rate (t=1.725, P=0.087), systolic blood pressure (t=1.367, P=0.179) or diastolic blood pressure (t=0.968, P=0.356) between two groups .

3.2. TGF-β1, Smad3 and IL-6 mRNA expression

In the persistent AF group, the TGF-β1, Smad3 and IL-6 mRNA expression semi-quantitative values were 1.5±0.4, 1.9±0.4 and 1.6±0.3, respectively; while in the paroxysmal AF group,the values were 1.3±0.3, 1.8±0.4 and 1.5±0.3 respectively. In the control group, the TGF-β1, Smad3 and IL-6 mRNA levels were 0.3±0.1, 0.5±0.2 and 0.4±0.1 respectively. TGF-β1, Smad3 and IL-6 mRNA levels in the paroxysmal AF group and the persistent AF group were significantly higher than the control group, the difference was statistically significant (P<0.05). There was no significantly difference in TGF-β1, Smad3 or IL-6 mRNA expression levels between the paroxysmal AF group and the persistent AF group, which was not statistically significant (P>0.05).

3.3. TGF-β1, Smad3 and IL-6 expression in protein levels

TGF-β1, Smad3 and IL-6 protein level semi-quantitative values of the persistent AF group, the paroxysmal AF group and the control group were shown in Table 1. TGF-β1, Smad3 and IL-6 protein levels in the paroxysmal AF group and the persistent AF group were significantly higher than the control group, the difference was statistically significant (P<0.05). There was no significant difference in TGF-β1, Smad3 or IL-6 mRNA6 protein levels between the paroxysmal AF group and the persistent AF group, which was not statistically significant (P>0.05).

Table 1

TGF-β1, Smad3 and IL-6 expressions of patients of each group in protein levels.

Groups	n	TGF-β1	Smad3	IL-6
Persistent AF group	37	2.3±0.8*	1.6±0.3*	1.9±0.4*
Paroxysmal AF group	13	2.1±0.7*	1.5±0.3*	1.8±0.4*
Control group	30	0.5±0.2	0.8±0.2	0.3±0.1

Note: Compared with normal control group * P<0.05.

3.4. Relationship of TGF-β1, Smad3 and IL-6 mRNA level expressions and clinical features of patients in the persistent AF group

The relationship of TGF-β1, Smad3 and IL-6 mRNA expression levels in patients with persistent AF and gender, age, NYHA classification, LAD, LVEF, resting heart rate, systolic blood pressure and AF time were shown in Table 2. TGF-β1 mRNA expression in ≥50 years subgroup was significantly higher than that in the <50 years subgroups, and it was higher in the NYHA Ⅲ subgroup than in the Ⅰ/Ⅱ grade subgroup, and it was also higher in the LVEF<50% subgroup than in LVEF≥50% group, and significantly higher in the AF time ≥36 months subgroup than that in <36 months subgroup, the differences were statistically significant (P<0.05). Smad3 and IL-6 expressions in the in the LVEF<50% subgroup were both higher that than that in LVEF≥50% group, and higher in the AF time ≥36 months subgroup than that in <36 months subgroup, the differences were statistically significant (P<0.05).

Table 2

Relationship of TGF-β1, Smad3 and IL-6 mRNA level expression and clinical features.

Groups	n	TGF-β1	Smad3	IL-6
Gender				
Male	21	2.0±0.8	1.6±0.3	2.0±0.4
Female	16	2.2±0.8	1.5±0.3	1.8±0.3
Age (years)				
≥50	21	2.3±0.9	1.6±0.3	1.7±0.3
<50	16	1.8±0.5*	1.4±0.2	2.0±0.4
NYHA Grade				
Ⅰ/Ⅱ Grade	32	1.5±0.4	1.3±0.3	1.8±0.3
III Grade	5	2.9±1.1*	1.6±0.2	2.2±0.4
LAD(mm)				
≥40	32	2.2±0.8	1.4±0.2	2.0±0.4
<40	5	2.0±0.8	1.6±0.2	2.2±0.4
LVEF(%)				
≥50	27	1.7±0.6	1.0±0.5	1.7±0.3
<50	10	2.3±0.7*	1.8±0.3*	2.5±0.5*
Resting heart rate				
≥90	11	2.3±0.7	1.6±0.4	1.8±0.4
<90	26	2.0±0.8	1.3±0.2	1.9±0.3
Systolic blood pressure (mmHg)				
≥140	15	1.9±0.7	1.7±0.4	1.7±0.4
<140	22	2.2±0.8	1.4±0.2	2.0±0.4
AF time (month)				
≥36	27	2.3±0.9	1.8±0.4	2.4±0.6
<36	10	1.7±0.6*	1.3±0.2*	1.8±0.4*

Note: Compared with the same factors in the group * P<0.05.

3. 5. Correlation analysis of TGF-β1, Smad3 and IL-6

In the persistent AF group, the TGF-β1, Smad3 and IL-6 mRNA expression were analyzed by Pearson product-moment correlation. The result showed that there was positive correlation between TGF-β1 and Smad3 (r=0.687, P=0.027), there was positive correlation between TGF-β1 and IL-6 mRNA (r=0.547, P=0.034), and there was also positive correlation between Smad3 and IL-6 mRNA (r=0.823, P=0.014).

4. Discussion

AF is the most common sustained arrhythmia, the overall incidence rate was 0.4% and the incidence of AF increased with age. The AF frequency can reach from 300 to 600 beats/min, no only fast but also irregular,beats faster than common people and absolutely arrhythmia, the atrial lost their contractile function. Study in our country showed the AF prevalence was 0.77%, and the male prevalence of AF (0.9%) was higher than females (0.7%). Furthermore there is a close correlation of the prevalence of AF and coronary heart disease, hypertension and heart failure and other diseases, AF has become an important issue of public health[1]. Wijffels *et al*[2] first proposed the concept of atrial electrical remodeling, this view has become popular of the AF research. The "electric remodeling" and "atrial structural remodeling" of the AF have also been concerned in recent years. Atrial structural remodeling mainly presented as atrial fibrosis, myofibrillar dissolution, atrial muscle cell apoptosis. Atrial interstitial fibrosis plays an important role in the atrial structural remodeling process,mainly presented the increased collagen deposition of the interstitial, imbalance and disorder which lead to the inhomogeneity of atrial conduction, provided the pathological basis of AF[3,4].

TGF-β has three subtypes, which were TGF-β 1, 2 and 3. TGF-β 1, 2 contribute to the formation of scar tissue, while TGF-β 3 has the anti-scarring effect. TGF-β 1 is an important factor to promoting fibrosis. In the AF development process, TGF-β 1 can promote atrial fibrosis, inflammation, tissue remodeling and then lead to tissue fibrosis[5]. Smad is an important signal transduction molecules of the TGF-β downstream, different type Smad can mediated different signal transduction of the TGF-β family members. There are reports speculated that Smad3 play a role in fibrosis; TGF-β 1/Smad signaling pathway was significant in the process of atrial fibrosis[5,6]. Studies have shown that there was an association between AF and inflammation, manifested in the maintenance of AF substrate, and inflammation is also involved in atrial structural and electrophysiological remodeling[7,8]. Recent studies have found that the IL-6 concentration of patients with AF was significantly higher.In the exclusion of other factors, the IL-6 levels and the intensity of the inflammatory response in patients with AF were positively correlated. In this study, TGF-β 1, Smad3 and IL-6 expression levels in plasma were detected by using RT-PCR and ELISA method in patients with AF, to study the specific regulatory pathways of TGF-β 1, Smad3 and IL-6 which promote atrial fibrosis,and further elaborate the mechanism of atrial structural remodeling.

There were no statistically significant of the clinical data such as gender, age, NYHA Grade of patients in the AF group and the control group by analyzing the clinical data we obtained. Our results showed that in the persistent AF group, the paroxysmal AF group and the control group the expression levels of TGF-β 1 mRNA semi-quantitative values were 1.5±0.4, 1.3±0.3, 0.3±0.1, respectively; Smad3 mRNA the expression levels of TGF-β 1 mRNA semi-quantitative values in the three groups were 1.9±0.4, 1.8±0.4, 0.5±0.2, respectively; IL-6 mRNA the expression levels of TGF-β 1 mRNA semi-quantitative values in the three groups were 1.6±0.3, 1.5±0.3, 0.4±0.1, respectively. The TGF-β 1, Smad3 and IL-6 mRNA levels in the paroxysmal AF group and the persistent AF group were significantly higher than the control group. There was no significantly difference in TGF-β 1, Smad3 and IL-6 mRNA expression levels between the paroxysmal AF group and the persistent AF group. Detected by ELISA, the TGF-β 1, Smad3 and IL-6 expression of the AF group (persistent and paroxysmal) were higher than the control group, which confirmed the increase of TGF-β 1, Smad3 and IL-6 were related with AF, and may be involved in the development of AF.

Li[5] confirmed TGF-β 1/Smad pathways involved in atrial structural remodeling of the AF, which was involved in the occurrence and development of the AF. TGF-β 1 take part in the fibrosis of the liver, lung, kidney, pancreas and other organs, which have been extensively proven, and also plays an important role in the process of cardiac fibrosis[9,10]. The study found that TGF-β 1 expression was high in patients with persistent AF in the high age group (≥50 years) and the NYHA Ⅲ subgroup.The TGF-β 1, Smad3 and IL-6 were highly expressed in the LVEF <50% and AF time ≥ 36 months subgroup. The heart function declined with age,and the prevalence of AF were higher, which suggested that the formation and maintenance of AF may have some relevance with the high expression of TGF-β 1, Smad3 and IL-6. TGF-β 1/Smad signal transduction pathways and inflammation involved in the fibrosis of AF and atrial structural remodeling. TGF-β 1 have a strong chemotactic effect on fibroblasts and inflammatory cytokines. Smad3 can mediate TGF-β 1 in the cytoplasm transduction by phosphorylation; TGF-β 1 involved in the induction of IL-6 high expression through the mitogen-activated protein kinase, induces IL-6 expression by stimulating the influx of calcium,and can induce IL-6 high expression by inducing the expression of intracellular H_2O_2. Pearson product-moment correlation showed that there were a positive correlation between TGF-β 1 and Smad3 and IL-6 in the persistent AF group. Inflammation and fibrosis are two relatively independent processes, but may be involved in the structural remodeling of AF, and induced the occurrence of AF generation[11-14].

TGF-β1/Smad signal transduction pathways and inflammatory reactions play an important role in the occurrence and developmentof AF[15-23], but the exact mechanism still lack of experimental support. TGF-β1/Smad signal transduction pathways and inflammatory reactions play an important role in the occurrence and developmentof AF, but the exact mechanism still lack of experimental support.With the further research,it can provide new ideas for the prevention and treatment of AF and find a new target for effective treatment.

Conflict of interest statement

We declare that we have no conflict of interest.

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