Effects of recombinant human brain natriuretic peptide on plasma TGF-β1 and PDCD5 levels in heart failure

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Abstract: Objective To observe the levels of plasma TGF-β1 and PDCD5 in patients with decompensated heart failure and the influence of recombinant human brain natriuretic peptide (rhBNP) treatment on them. **Methods** 40 patients in NYHA functional classification IV (including 22males, 18 females, aged 65.0±3.7 years) were randomly divided into two groups. 20 patients in rhBNP-treated group received conventional drug such as diuretics, anticoagulation, angiotensin-converting enzyme inhibitor and rhBNP (1.5u g/kg bolus intravenous injection followed by 0.0075mg/kg/min/ intravenous drop infusion for 72 hours). 20 patients in control group received conventional drugs. The levels of plasma TGF-β1 and PDCD5 were determined before and after treatment by enzyme linked immunosorbent assay (ELISA). Results: There were significant differences between TGF-β1 levels among different time points in rhBNP-treated group (Ftime=585.044, P<0.001; Fgroup=40.767, P<0.001). And there were significant differences between PDCD5 levels among different time points in rhBNP-treated group (Ftime=23.615, P<0.001; Fgroup=35.654,P<0.001). **Conclusion** Long-term treatment with rhBNP decreases the levels of plasma TGF-β1 and PDCD5 persistently. RhBNP may be associated with the prevention of cardiac fibrosis and apoptosis in cardiomyocytes.

[Xianglin Lian, Guojie Yang, Lixin Yang, Zidi Chen, Haopeng Kang,Zhihong Ye, Zihan Wei, Peng Qin, Guodong Li, Dongbo Li. **Effects of recombinant human brain natriuretic peptide on plasma TGF-β1 and PDCD5 levels in heart failure.** *Life Sci J* 2013;10(2):1560-1563**].** (ISSN:1097-8135). http://www.lifesciencesite.com. 212

Keywords: rhBNP; Heart failure; TGF-β1; PDCD5

1. Introduction

Chronic heart failure is the leading cause of mortality and morbidity in developed and most developing countries. Cardiac ventricular remodeling and apoptosis were important pathophysiological process of heart failure. Cardiac remodeling is characterized by cardiomyocyte hypertrophy, cell loss and expression of extracellular matrix (ECM). The over-proliferation of Cardiac fibroblasts which leading to the expression of extracellular matrix induces myocardial fibrosis and cardiac remodeling finally. Numerous studies have emphasized the important role of transforming growth factor-β1 (TGF-β1) which is dispersed distribution and has myocardial fibrosis aggravated. Programmed cell death5(PDCD5)antibody is closely related to the development of a number of apoptosis abnormal diseases. The level of plasma PDCD5 can reflect the degree of cardiomyocyte apoptosis.

B-type natriuretic peptide (BNP) is a cardiac hormone produced primarily by ventricular myocytes. BNP not only possesses potent natriuretic, diuretic, and lusitropic properties, but also counteracts angiotensin actions, inhibits renin and aldosterone release, and has direct and indirect antifibrotic properties. Plasma concentrations of BNP are markedly elevated in patients with congestive heart failure and acute myocardial infarction, and it is now a valuable maker of heart failure.

Recombinant human brain natriuretic peptide (rhBNP) is one of new drugs in the medical management of heart failure. RhBNP is a man-made peptide through gene engineering, which has the same sequence and structure of amino acid as brain natriuretic peptide (BNP). Recent clinical and experimental studies have indicated that rhBNP could greatly improve symptoms and hemodynamic parameters of acute heart failure in the initial stage. However, little is known about the curative effect of rhBNP in patients of decompensated heart failure. Moreover, it is obscure that the effect of rhBNP in cardiac remodeling. We will observe the change of vital sign as well as plasma TGF-β1 and PDCD5 antibody in patients with decompensated chronic heart failure and the influence of rhBNP on them,and it will also lay a fundamental ground on the development of novel methods to resist cardiac remodeling and to protect heart function.

2. Data and methods

2.1 Subjects

Before the initiation of any study procedures,

a written informed consent was obtained from each study participant. The ethics committee of Zhengzhou University approved the study. The study was designed to be a nested case-control study within a prospective cohort of 40 patients in NYHA functional class Ⅳ (including 22males, 18 females, aged 65.0±3.7 years) hospitalized in the department of geriatric cardiology from 2010 to 2011.

All patients had established heart failure and none had diabetes, hyperlipidemia, liver disease, renal impairment, pulmonary fibrosis and chronic obstructive pulmonary disease, extensive wounds, metabolic bone disease, malignancy, connective tissue disorders, chronic or acute inflammatory disease, or recent surgery were excluded from the study. Furthermore, patients over 80 years old and patients with a pacemaker/implantable cardioverter-defribrillator were also excluded.

In order to rule out conditions potentially associated with heart failure (such as cardiogenic shock, contraindications to vasodilators, rupture of the heart, rupture of papillary muscle, cardiac aneurysm, the allergy to rhBNP, hepatic failure or kidney failure, serious heart valves stenosis, hypertrophic cardiomyopathy or restrictive cardiomyopathy as well as constrictive pericarditis), a standardized protocol including a detailed history, transthoracic echocardiography, exercise test and any other indicated test, was performed. The transthoracic echocardiogram was carried out using LOGIQ7 echocardiographic equipment.

The subjects in this prospective, double-blinded placebo-controlled trial were randomized into two groups: rhBNP-treated group and the control group. Each group consists of 20 cases. Patients in control group received conventional drug, such as diuretics, anticoagulation, angiotensin-converting enzyme inhibitor (ACEI) and plus nitroglycerin (initial dose of 5 μg/min, with subsequent dose increment of 5μg/min in every 3 to 5 minutes). Patients in rhBNP-treated group were assigned to plus rhBNP (1.5μg/kg bolus intravenous injection and maintained persistently at a dosage of 0. 0075 mg/kg/min/intravenous drop infusion for 72 hours) based on the control group received.

2.2 Laboratory methods and blood sampling

Blood samples, for the measurement of TGF-β1 and PDCD5 antibody, were obtained by a peripheral vein draw and within an hour after collection they were centrifuged at 3,200 × g for 10 minutes at a temperature of about 4°C. The serum was separated into aliquots and was stored in -80°C until personnel blinded to the patients' clinical information performed the assay analysis. Serum levels of TGF-β1 and PDCD5 antibody were determined at baseline before drug bolus (time 0), and at 7, 14, 30d (24h after cessation of the infusion) by commercial standardized in vitro enzyme-linked immunosorbent assay (ELISA) methods according to the manufacturer instructions (ZSGB-Bio, Beijing, China).

2.3 Statistical Analyses

All values were expressed as mean±standard error . The statistical analysis on differences among the groups was done by uni-variate test of repetitive measure ANOVA. Differences were judged to be significant when $P<0.05$. Statistical analyses were performed using SPSS 17.0 statistics software.

3. Results

3.1 The changes of TGF-β1 levels: There were significant differences in the value of TGF-β1 among different time points in rhBNP-treated group (table1, Ftime=585.044, P<0.001; Fgroup=40.767, P<0.001). The values of TGF-β1 in rhBNP-treated group were lower than that in control group(P<0.001). There were no significant difference of the value of TGF-β1 among different time points in control group(P TGF-β1=0.143).

3.2 The changes of PDCD5 antibody levels : There were significant differences in the value of PDCD5 antibody among different time points in rhBNP-treated group (table 1, Ftime=23.615, P<0.001; Fgroup=35.654, P<0.001). The values of PDCD5 antibody in rhBNP-treated group were lower than them in control group(P<0.001). There were no significant difference of the value of PDCD5 antibody among different time points in control group(P PDCD5 =0.28).

Table 1 The levels of TGF-β1 and PDCD5 of two groups at different time points

groups	n	ρ(TGF-β1)/ (ng•L^{-1})	ρ(PDCD5)/ (ng•L^{-1})
rhBNP-treated group			
T0	20	329.74±84.77	3.51±1.33
T1	20	169.38±33.07*	3.41±1.25*
T2	20	155.05±19.31*	2.64±1.07*
T3	20	134.12±24.15*	2.52±1.21*
control group			
T0	20	332.34±78.39	3.45±1.81
T1	20	259.03±31.12	3.42±1.42
T2	20	270.08±31.53	3.41±1.51
T3	20	283.06±29.59	3.36±1.32
F group(*P*)		40.767(<0.001)	35.654(<0.001)
F time(*P*)		585.044(<0.001)	23.615(<0.001)
F interaction(*P*)		16.067(<0.001)	14.408(<0.001)

* *P* value<0.05vsT0 rhBNP-treated group

4. Discussion

Ventricular remodeling is very important in the genesis and development of heart failure, myocardium fibrotic and apoptosis are important divisions of heart failure [1]. When heart disease leads to major health problems, the application of medicine for dilating blood vessels, diuretics and cardiac glycoside may alleviate the symptoms of heart failure in patients, but, they cannot reverse the trend of myocardium fibrotic and apoptosis because of the activation of a series of neuroendocrine system, such as RAAS. Recurring attacks of cardiac dysfunction or acute decompensation can lead to chronic heart failure. Patients requiring frequent visits to hospital or even result in sudden death. Hence, the focus of heart failure treatment is the antagonism of over-activation of neuroendocrine system and cardiac remodeling in addition to improving the blood flow dynamics.

B-type natriuretic peptide (BNP) is a kind of natural RAAS antagonistic hormone. Through dilating blood vessels selectively and sodium and urine excretion, BNP can quickly correct hemodynamic disorder. Meanwhile, BNP can act against the over-activation of neuroendocrine system and heart remodeling. What is more, it can improve heart function [2]. RhBNP is made from genetic recombination and is a kind peptide of freeze-dried biological agent which has the same amino acid sequence and solid structure as the endogenous BNP of humans[3]. Combined with natriuretic peptide receptor embedded in effector cell membrane, rhBNP can activate the combination of sweet bird cyclase which is connected to it and enhances the levels of cyclic guanosine monophoshate in effector cells. The elevated cGMP acts on the protein kinase G on the capillary endothelial cell membrane, which makes myosin light chain dephosphorylated, vascular smooth muscle contraction and relaxation and extended vessels[4]. In addition, the study shows that rhBNP can produce antagonistic plasma aldosterone and norepinephrine endothelin, and other neurohumoral shrinkage in blood vessels. Meanwhile, it can decrease myocardial apoptosis. Hence, simulating physiological process, rhBNP can play the same important role in resisting the over-activation of neuroendocrine system and ventricular remodeling as endogenous BNP [5].

Watkins and other researchers found that TGF-β1 plays an important role in myocardial cell hypertrophy and heart interstitial proliferation [7]. According to the study, increasing the level of serum TGF-β1 can increase the incidence of fatal cardiovascular events significantly, such as accelerating myocardial fibrosis, ventricular cardiac and ventricular fibrillation. So, serum TGF-β1 is one risk factor of sudden cardiac death [7-9]. Acting on CGMP/PKG, PKG and MEK/ERK and other signal pathways, BNP can prevent serum TGF-β1 and induce its fibrosis. Meanwhile, the deterioration of cardiac function is alleviated. It has been observed that the serum TGF-β1 level of heart failure patients reduced significantly at different points after the treatment by rhBNP. However, the serum TGF-β1 level of heart failure patients with conventional treatment has no obvious changes at different times [10]. Hence, rhBNP may lower the serum TGF-β1 level to inhabit cardiac fibrosis.

Myocardial apoptosis plays an important role in the pathogenesis of the heart failure disease. PDCD5 is a newly discovered gene concerned with cell apoptosis and highly expressed in the process of cell apoptosis. It translocates from cytoplasm into nucleus and promotes cell apoptosis[11,12]. Our results reveal that the levels of PDCD5 antibody in rhBNP-treated groups were significantly reduced at different points after the treatment by rhBNP. However, the PDCD5 level of heart failure patients with conventional treatment has no obvious change at different times. So rhBNP may reduce the level of PDCD5 antibody so as to inhibit the myocardial apoptosis.

In summary, our experiment results showed that the treatment with rhBNP can better improve the symptoms of heart failure than the conventional treatment. The long-lasting effect of rhBNP which decreases the levels of plasma TGF-β1 and PDCD5 antibody may be associated with resisting cardiac fibrosis, myocardial cell apoptosis and other way of inhibiting ventricular remodeling, apart from avoiding retention of sodium and water.

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21/5/2013