Klotho protein lowered in senile patients with brady sinus arrhythmia

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Abstract: Objective: To explore the correlationship between brady sinus arrhythmia and the levels of serum klotho protein in aged. Methods: 104 patients over 75 years old with brady sinus arrhythmia (experiment group) were enrolled, including 34 cases of sinus arrest, 43 cases of sinus bradycardia and 25 cases of atrioventricular block. 109 patients over 75 years old without brady sinus arrhythmia were chosen as control group. All subjects were monitored by Holter. The levels of serum klotho protein were detected and compared among three groups. The correlation between the frequency of sinus arrest and the levels of serum klotho protein was analyzed simultaneously. Results: The levels of serum klotho protein in experiment group were lower than that in control group (P<0.01); the sinus arrest frequency was negatively correlated with the levels of serum klotho protein. The levels of serum klotho protein in patients with sinus arrest were lower than that with sinus bradycardia and atrioventricular block (P<0.05). But there was no significant difference between sinus bradycardia group and atrioventricular block group. Conclusion: The levels of serum klotho protein may reflect the function of sinoatrial node and could be used as an index to estimate the function of sinoatrial node.

Keywords: Senile, brady sinus arrhythmia, klotho

Introduction

With human aging, degenerative changes happen in body organs in no exception of cardiac conduction system. Sinus node, the headquarters of conduction system, gives off fewer and fewer signals, which leads to sinus bradyarrhythmia and sinus arrest. Study showed that klotho was vital to aging and solely expressed in sinus node in heart, which plays a protecting role to pacemake cells [1-3]. This study focused on the senile patients with bradyarrhythmia, who was over than 75 years old, in order to know the relationship between klotho protein and bradyarrhythmia.

Materials and methods

Materials

213 cases, ranged from July 2010 to June 2013, provided by The Department of Geriatric of The First Affiliated Hospital of Xi’an Jiaotong University School of Medicine. All of them volunteered to participate in this study. They were divided into experiment group and control group. The former included 104 patients with sinus bradycardia (heart rate ≤50 bpm) diagnosed by rest electrocardiogram, the control group had 109 cases with normal heart rate. All cases had checked the 24 hours electrocardiogram (ECG). The general parameters of the two groups see Table 1.

The standard admission for subject as following: ① over 75 years old; ② hospital patients for the first time; ③ without chronic or acute liver, kidney diseases and tumor; not complicated with cerebral or peripheral vascular diseases.

Methods

Ambulatory ECG monitoring: 24 hours electrocardiogram (Vaso-Medical BIOX-200) was used in this study. The results from Ambulatory ECG monitoring were analyzed by two cardiologists.

The determination of serum klotho: The supernatant of specimen, 3~5 mL, which collected
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from elbow venous blood of cases, were centrifuged under 2000 r/min for 10 min and stored at -80°C. Klotho Protein Detection: using ELISA method. Diluting the supernatant of specimen according to 1:10 and then adding it to 96-wells plate, each well 0.1 mL, 4°C overnight. The next day, discarding all the liquids of 96-wells plate and washing five times with the Tween-20 PBS, after then, adding freshly prepared TMB to the plate, each well 0.1 mL. Finally, putting the plate into 37°C incubator for 30 min and then adding stop solution to terminate the reaction. The values of absorbance were measured by microplate reader (Model 550, produced by United States) and repeatedly tested for at least 2 times. NO Determination: employ nitroreductase method. The kits were provided by Southern built Bio Co., Ltd. The steps were strict accordance with the instructions. The results were detected by UV-Vis spectrophotometer (model SUV-2120) of Korean Scinco, and calculated according to formula from instructions.

**Statistical analysis**

All Statistical analyses were performed with SPSS17.0/PC Package, and all values were expressed as mean ± standard deviation (X ± s). The comparisons between the two groups were made by using Student’s T-test, and the criteria for significance difference was P<0.05.

**Results**

The results of ambulatory ECG monitoring

The experiment group was subdivided into three subgroups on the records of ambulatory ECG monitoring, they were sinus bradycardia group (SB, n=43), sinus arrest group (SA, n=36) and atrioventricular block group (AB, n=25).
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Patients with coexisting heart rhythm (for instance all kinds of premature, paroxysmal atrium fibrillation and so on) were recorded as basic heart rhythm. When sinus bradycardia, sinus arrest and atrioventricular block were presented in one record simultaneously, it was classified into SA group. When sinus bradycardia and atrioventricular block emerged in one record, it should be classified into SA group.

Levels of serum klotho in different groups

Klotho protein absorbance were lower in bradycardia group than that in control group. There was statistically significant between two groups (P<0.01). In the subgroups, klotho protein absorbance is lower in SA group than that in the other two subgroups (SB and AB group), there was also statistically significant among them (P<0.01). See Table 2.

Correlation between frequency of sinus arrest and serum levels of klotho

The correlation research between frequency of sinus arrest and serum levels of protien klotho showed that klotho absorbance decreased with increasing frequency of sinus arrest (r=-0.8884, P<0.05). Figure 1.

Discussion

Klotho is a newly identified anti-aging gene in 1997 by Kruo in the study of relationship between spontaneous hypertension and aging [1]. The gene consists of 5 exons and 4 introns in chromosome 13 in humans [4, 5]. The gene encodes secreted transmembrane protein. The mechanism now is elucidated by researchers as following: when transcriptional termination site located in upstream of exon 4 of the membrane form accepts an insertion sequence, the product is secreted form with 5.8 kbp in length and composed of exons 1, 2 and long exon 3 [6]. Recently, a number of profound researches focus on the structure, expression, product and function of klotho. Takeshita K [3] found that klotho plays a protecting role in sinus node function, its specific mechanism is not to be elucidated yet.

Serum klotho protein absorbance was lower in the sinus bradycardia group than those of control group, the difference is statistically significant (P<0.01). Serum klotho protein absorbance is also lower in sinus arrest group than that in sinus bradycardia and atrioventricular block group (P<0.05). Further analysis found that the serum klotho protein absorbance showed negative relationship with frequency of sinus arrest (r=-0.8884, P<0.05). It revealed that serum klotho protein played an important role in maintaining normal sinoatrial node function. As human body aging, because of the concentration of serum klotho protein decreasing, the protecting role of klotho is getting weaker and weaker. This may be the reason that brady sinus arrhythmia occurs easily in elderly patients.

This study suggested that the serum concentration of klotho protein might reflect the function state of sinoatrial node in the patients with sinus brady-arrhythmia, especially in the patients with sinus arrest. Further study should focus on how klotho protein exerts its protecting role on sinoatrial node function.

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Disclosure of conflict of interest

None.

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