Disturbed expression and distribution of myocardial connexin 43 in sudden death patients with hyperthyroid heart disease

Bo Su1*, Yao Chen2*, Aimin Du3

1Department of Pathology, School of Medicine, Yangtze University, Jingzhou, Hubei Province, China; Departments of Ophthalmology, 2Endocrinology, The Central Hospital, Jingzhou, Hubei Province, China. *Equal contributors.

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Abstract: This study aimed to investigate the relationship between the expression and distribution of myocardial connexin 43 (Cx43) in the left ventricle and the incidence of sudden death due to hyperthyroid heart disease. Total 27 autopsy cases were selected from the Pathology Department of Medical School, Yangtze University from 2009 to 2014. The cases were divided into three groups (n=9): Experimental group, 6 males and 3 females, age range 20-57 years old, suddenly died of hyperthyroid heart disease; Hyperthyroid group, 7 males and 2 females, age range 27-63 years old; Control group, 5 males and 4 females, age range 27-49 years old. After pathological examination, Cx43 in the left ventricular muscle of each case was detected with immunohistochemical staining. In experimental group, Cx43 positive staining was found in the cytoplasm or side-side connection of ventricular myocardial cell, and the positive area and average optical density of Cx43 positive particles significantly decreased. In hyperthyroid group, average optical density of Cx43 positive particles decreased. In control group, Cx43 positive staining was found in the intercalated disc. In conclusion, the expression of myocardial Cx43 significantly decreased in sudden death patients with hyperthyroid heart disease. Our findings suggest that decreased expression of Cx43 may be related to sudden death of hyperthyroid heart disease.

Keywords: Connexin 43, hyperthyroid heart disease, immunohistochemistry, pathology, sudden death

Introduction

Myocardial hypertrophy often leads to irreversible changes in cardiac structure and is the independent risk factor of the mortality of cardiovascular diseases [1]. In recent years, the role of junction protein (Connexin, Cx), especially connexin 43 (Cx43), in sudden cardiac death and cardiomyopathy has gain much attention [2-4]. However, the relationship between Cx43 expression and sudden death of hyperthyroid heart disease has not been reported in the literature. Therefore, this study aimed to investigate the relationship between the expression of Cx43 in the left ventricle and the incidence of sudden death due to hyperthyroid heart disease.

Materials and methods

Samples

27 cases were selected from the samples kept at Pathological Department, School of Medicine of Yangtze University from 2009 to 2014, with complete medical records, autopsy records, pathology and autopsy report, and were divided into three groups: (1) the hyperthyroid heart disease sudden death group (experimental group): 9 cases including 6 males and 3 females; aged 20-57 years, with average 36.4, all died of hyperthyroid heart disease only, and met the following criteria: left ventricular wall thickness ≥1.2 cm; the heart weight ≥400 g; (2) hyperthyroid group: 9 cases including 7 males and 2 females; age 27-63 years old, average age 43.6; (3) non-cardiac death group (control group): 9 cases including 5 males and 4 females; age 27-59 years old, average age 41.3; met the following criteria: did not suffer from hyperthyroidism, the left ventricular wall thickness <1.2 cm; the heart weight <350 g; 8 cases died from craniocerebral trauma and 1 case died from acute severe hepatitis.
Table 1. Heart parameters in three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Average heart weight (g)</th>
<th>Average left ventricular wall thickness (cm)</th>
<th>Myocardial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td>513&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.47&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>Hypertrophy</td>
</tr>
<tr>
<td>Hyperthyroidism group</td>
<td>300</td>
<td>1.14</td>
<td>Normal</td>
</tr>
<tr>
<td>Control group</td>
<td>285</td>
<td>1.00</td>
<td>Normal</td>
</tr>
</tbody>
</table>

All cases met the following exclusion criteria: related diseases out of hypertensive heart disease, cardiomyopathy, coronary artery stenosis and myocardial infarction in myocardial damage.

Three groups of bodies were kept at 4°C within 24 h after death, dissected within 24 h-72 h, hearts were removed and fixed in 10% formalin. Each heart specimen was weighed, left ventricular wall thickness was measured, left and right coronary artery and its branches were observed. The anterior walls of the left ventricle were embedded in paraffin, sliced into sections of 4 μm thickness, and stained by hematoxylin and eosin.

Immunohistochemical analysis

Cx43 was detected by immunohistochemical staining as described previously [5].

Briefly, following deparaffinization in xylene and dehydration in graded ethanol solutions, the sections were heated at 121°C for 20 min in for antigen retrieval. Endogenous peroxidase activity was blocked via the incubation of the sections in 30 ml/l hydrogen peroxide for 20 min. The tissue sections were incubated with a primary rabbit anti-Cx43 monoclonal antibody (Catalog No. 71-0700; dilution 1:200; Invitrogen Corporation, USA) at 4°C overnight, and stained using the labeled streptavidin-biotin method. For negative controls of immunohistochemical staining, PBS was used instead of primary antibody. Lymphocytes were used as the positive control. All slides were assessed independently by two pathologists, who were blinded to the clinicopathological data. Image-ProPlus6.0 system was applied to analyze Cx43 expression. 5 fields were randomly selected; Cx43 positive area (S value) and the average optical density (OD) were measured.

Statistical analysis

Data were reported as the $\overline{x} \pm s$. Differences between groups were analyzed by Student’s unpaired t test or analysis of variance followed by Tukey’s post hoc test. Statistical significance was considered when $P$ was less than 0.05. Statistics software SPSS 18.0 was used for statistical analysis.

Results

Cardiac lesions in three groups

The parameters of the hearts in three groups were summarized in Table 1. In experimental group, the average weight of the heart was 513 g (range 410 g-680 g). Gross appearance: the heart increased slightly, apex was obtuse, the average of left ventricular wall thickness was 1.47 cm (range 1.30 cm-2.20 cm), left and right coronary arteries had patency, no obvious atherosclerotic plaque or thrombosis was observed. Under the microscope: some myocardial cells thickened; the nuclei increased with round or oval shape, hyperchromatic nuclei, there were 3 cases with myocardial interstitial fibrosis.

In hyperthyroidism group, the average weight of the heart was 300 g (range 250 g-321 g). Gross appearance: the enlargement of the heart was not obvious, the average of left ventricular wall thickness was 1.14 cm (range 0.90 cm-1.17 cm); no obvious atherosclerotic plaque and thrombosis was observed. Under the microscope: the structure of myocardial cells showed no obvious abnormalities.

In control group, the average weight of the heart was 285 g (range 185 g-300 g). Gross appearance: the enlargement of the heart was not obvious, the average of left ventricular wall thickness was 1.00 cm (range 0.80 cm-1.10 cm); no obvious atherosclerotic plaque or thrombosis was observed. Under the microscope: the structure of myocardial cells showed no obvious abnormalities.

Immunohistochemical analysis of myocardial Cx43 in three groups

In control group, Cx43 positive granules were brown and distributed in the ventricular myocytes of the end-to-end connection as well as vertical and longitudinal myocardial cells in intercalated disc area (Figure 1A). In hyperthyroidism group, Cx43 positive granules were
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stained light, located in the intercalated disks, a few scattered in the cells (Figure 1B). Compared with control group, in experimental group the number of Cx43 positive granules significantly reduced (Figure 1C). In experimental group, Cx34 positive granules were scattered in the cells (Figure 1D), or were distributed in the cytoplasm parallel to cell axis (Figure 1E), or distributed in the cell lateral connections (Figure 1F).

S value and OD value of Cx43 staining in each group were analyzed. Compared with control group, S value was significantly lower in experi-

Figure 1. Immunostaining of Cx43 in three groups. A. Control group, strong Cx43 positive granules were distributed in the intercalated disks. B. Hyperthyroid group, moderate positive Cx43 granules were distributed. C. Experimental group, weak positive Cx43 granules were distributed. D. Experimental group, Cx43 positive granules were distributed in the cells. E. Experimental group, Cx43 positive granules were distributed in parallel with the long axis myocardial cells. F. Experimental group, Cx43 positive granules were distributed in side-side junctions. Magnification, ×400.
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Myocardial cell electric coupling is mediated by gap junction (GJ) which is mainly distributed in the intercalated disks [6]. GJ channel is composed of two connected bodies docking in cell membrane. Each connecting body is composed of 6 subunits of connexins (Cx), which form a narrow hole to allow small molecule less than 1 kD ion current. Human myocardial cells mainly express three forms of Cx including Cx40, Cx43 and Cx45, and Cx43 is the main form. The normal distribution and expression of Cx43 is important for normal cardiac electrical activity and the coordination of contraction. Peters et al. found that abnormal Cx43 distribution was part of the early remodeling of myocardium after infarction that causes ventricular tachycardia [7].

This study focused on Cx43 expression of left ventricular muscle obtained from thyrotoxic heart failure death. By immunohistochemical analysis, Cx43 positive particles of control group were uniformly colored and distributed in the intercalated disks. Cx43 positive granules of hyperthyroidism group were stained moderately and mostly distributed in the intercalated disks, a few Cx43 positive granules were scattered in myocardial cells. Cx43 positive granules of experiment group decreased significantly and were unevenly distributed. Statistical analysis showed significant differences in Cx43 staining including positive area and mean optical density among experimental group, hyperthyroidism group and control group. These data suggest that the distribution of Cx43 changes significantly in patients of sudden death due to hyperthyroid heart disease.

Long term hormone stimulation can cause myocardial hypertrophy and ventricular remodeling [8-13]. Myocardial interstitial fibrosis resulted in the extrusion and package of Cx43. In hyperthyroid heart disease patients with myocardial hypertrophy, increased myocardial intracellular Ca²⁺ influx would activate proteinase calpains, resulting in reduced Cx43 [14]. In addition, myocardial ischemia caused by oxygen demand of myocardial hypertrophy increased Cx43 degradation [15].

In summary, in the patients with hyperthyroid heart disease abnormal myocardial end-to-end connection mode of Cx43 distribution could lead to the changes in intercellular coupling and the decrease in longitudinal velocity, and induce the occurrence of arrhythmia and sudden death.

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Disclosure of conflict of interest

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Address correspondence to: Aimin Du, Department of Endocrinology, The Central Hospital, Jingzhou, China. E-mail: 7885909@qq.com

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