Expression of CX43 and SOCS-1 in patients with sudden cardiac death

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Abstract: Background: This research was aimed to study the expression of CX43 and SOCS-1 in cardiac tissues of patients with sudden cardiac death (SCD). Method: From March 2013 to March 2015, 21 cardiac tissue specimens from patients with SCD as well as cardiac tissue specimens from non-SCD patients were collected. RT-PCR and immunohistochemical staining were applied to detect mRNA and protein expression of CX43 and SOCS-1 in the cardiac tissues, respectively. Result: Relative expression of CX43 and SOCS-1 in the cardiac tissues from patients with SCD was 0.395+0.014 and 0.823+0.021, respectively, vs. 0.892+0.103 and 0.429+0.037 in the non-SCD patients (P<0.05). The positive area (S value) of immunohistochemical staining and OD value of CX43 in SCD patients were 1.538+0.328 and 0.42+0.003, respectively, with a significant decline as compared with those in non-SCD patients, which were 2.402+0.928 and 1.421+0.007, respectively (P<0.05). The S value of immunohistochemical staining and OD value of SOCS-1 in the SCD patients were 2.231+0.515 and 1.129+0.002, respectively, significantly higher as compared with those in non-SCD patients (1.369+0.257 and 0.201+0.005) (P<0.05). Conclusion: Downregulation of CX43 and upregulation of SOCS-1 were the risk factors of SCD.

Keywords: Sudden cardiac death, suppressor of cytokine signaling-1 (SOCS-1), connexin-43, immunohistochemistry

Introduction

Sudden cardiac death (SCD) is an unexpected death due to cardiac causes occurring in a short time period (generally within 1 h of symptom onset) [1]. Epidemiological study has shown that the incidence of SCD is increasing as more and more people are suffered from cardiovascular diseases [2]. Since SCD is associated with multiple factors and the molecular mechanism of SCD remains unclear, the diagnosis and prevention of SCD are generally based on the criteria for primary diagnosis. No independent diagnostic plan is yet available for SCD [3, 4]. Suppressor of cytokine signaling-1 (SOCS-1) is a novel therapeutic target for cardiac injury. It is produced through the induction of cytokines and regulates cytokine expression via negative feedback. SOCS-1 is upregulated in a variety of cancers [5]. CX43 is an important gap junction protein [6, 7]. This study compared the mRNA and protein expression of CX43 and SOCS-1 in cardiac tissues from 21 SCD patients and 21 non-SCD patients. We aimed to reveal the molecular mechanism for the prevention and diagnosis of SCD.

Materials and methods

General data

Cardiac tissues from 21 SCD patients and 21 non-SCD patients were collected by autopsies at the pathology laboratory of Second Affiliated Hospital of Xi’an Jiaotong University from March 2013 to March 2015. The study was approved by the ethics committee of Second Affiliated Hospital of Xi’an Jiaotong University.

Reagents

Tissue RNA extraction kit (Tiangen Biotech (Beijing) Co., Ltd.), reverse transcription kit (Fermentas), real-time quantitative fluorescence PCR kit (ThermoFisher), rabbit polyclonal antibodies against CX43 and SOCS-1 (Santa Cruz), goat anti-rabbit polyclonal antibodies (Shanghai
CX43 and SOCS-1 with SCD

Table 1. Primer design

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5’-3’)</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOCS-1</td>
<td>F: AGCCCTTTCCCGGGCTCTCA</td>
<td>243</td>
</tr>
<tr>
<td></td>
<td>R: GTTACGCTCCAGTGACAGAC</td>
<td></td>
</tr>
<tr>
<td>CX43</td>
<td>F: GCAGCCATGGGCGTGTGATAG</td>
<td>380</td>
</tr>
<tr>
<td></td>
<td>R: ATGTCAAGTGTGCTTCTGGA</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>F: AAATCTGGCCTGTGGATCGGA</td>
<td>615</td>
</tr>
<tr>
<td></td>
<td>R: TCAAGTGGGGGACAAAG</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Comparison of relative mRNA expression of CX43 and SOCS-1 between the two groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>N/case</th>
<th>CX43</th>
<th>SOCS-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCD patients</td>
<td>21</td>
<td>0.395±0.014</td>
<td>0.892±0.103</td>
</tr>
<tr>
<td>Non-SCD patients</td>
<td>21</td>
<td>0.823±0.021</td>
<td>0.429±0.037</td>
</tr>
<tr>
<td>t</td>
<td>--</td>
<td>19.336</td>
<td>18.267</td>
</tr>
<tr>
<td>P</td>
<td>--</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Statistical analysis

SPSS 19.0 was used for statistical analysis. The mRNA and protein expressions of CX43 and SOCS-1 were compared between the two groups by using paired t-test. P<0.05 indicated significant difference.

Results

mRNA expression of CX43 and SOCS-1

The mRNA expression of CX43 was significantly lower in the cardiac tissues of SCD patients compared to those of the non-SCD patients (P<0.05) (Table 2 and Figure 1A). The mRNA expression of SOCS-1 in the cardiac tissues of the SCD patients was also significantly lower as compared with those of the non-SCD patients (P<0.05) (Table 2 and Figure 1B).

Protein expression of CX43 and SOCS-1

S and OD value of CX43 in the left ventricle of SCD patients showed a significant decrease as compared with that of the non-SCD patients (P<0.05) (Table 3; Figure 2A and 2B); however, the S and OD value of SOCS-1 in the left ventricle of SCD patients were significantly increased as compared with that of the non-SCD patients (P<0.05) (Table 3; Figure 2C and 2D).

Discussion

The S and OD value of CX43 mRNA expression were significantly lower in the SCD patients as compared with that of the non-SCD patients (P<0.05). This indicated the downregulation of CX43 in SCD. Gap junction (GJ) channel plays an important role in function execution in cardiac cells by transmitting the electric coupling signals and intercellular chemical/metabolic signals. As a key component of the GJ channel, CX43 is highly expressed in the ventricular myocytes [8]. Severs NJ [9] and Tribulova N et al. [10] pointed out that the expression, localization and phosphorylation of CX43 in the ventricular myocytes all had an impact on GJ channel in the myocytes, thus contributing to cardiac diseases. Moreover, Rhett JM et al. [11] showed that CX43 was involved in the pathogenesis of cardiac diseases through GJ channel, but also facilitated the onset through hemichannels.

Deficiency of blood and oxygen supply to the myocytes is among the major cases of myocyte...
CX43 and SOCS-1 with SCD

Figure 1. mRNA expression of CX43 and SOCS-1 and in the two groups. 1 stands non-SCD patients group; 2 stands SCD patients group.

Table 3. S values and OD values of CX43 and SOCS-1 in the left ventricle in the two groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>N/case</th>
<th>CX43</th>
<th>SOCS-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S</td>
<td>OD</td>
</tr>
<tr>
<td>SCD patients</td>
<td>21</td>
<td>1.538±0.328</td>
<td>0.42±0.003</td>
</tr>
<tr>
<td>Non-SCD patients</td>
<td>21</td>
<td>2.402±0.928</td>
<td>1.421±0.007</td>
</tr>
<tr>
<td><em>t</em></td>
<td></td>
<td>132.024</td>
<td>25.127</td>
</tr>
<tr>
<td><em>P</em></td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Figure 2. Immunohistochemical staining of CX43 and SOCS-1 in the cardiac tissues (400 ×). A: CX43 expression in the cardiac tissues of the SCD patients; B: CX43 expression in the cardiac tissues of the non-SCD patients; C: SOCS-1 expression in the cardiac tissues of the SCD patients; D: SOCS-1 expression in the cardiac tissues of the non-SCD patients.

CX43 is involved in the pathogenesis of cardiac diseases by being part of the GJ channels and hemichannels. Along with myocyte injury in cardiac disease, CX43 is usually downregulated in the myocytes, thus aggravating myocyte injury.

We found that the S and OD value of SOCS-1 mRNA expression in 21 SCD patients were significantly higher as compared with 21 non-SCD patients (*P*<0.05). Thus SOCS-1 protein was upregulated in SCD. SOCS family proteins are composed of an N-terminal region of variable length and amino acid composition, a central SH2 domain, and a previously unrecognized C-terminal motif [14]. SOCS-1 is an important SOCS protein and is generated by the induction of IL-6, IL-4, IL-3 and (IFN)-γ [15]. Besides, SOCS-1 plays a negative feedback regulation role in the cytokines through the interaction with the JAK signaling pathway. Clinical studies [16] have shown that IL-6 expressed by the myocytes can activate the JAK/STAT signaling pathway and MAPK signaling pathway by binding with the receptors. The pathogenesis of myocardial hypertrophy [17-19] involves the working of JAK/STAT pathway, MAPK pathway...
and CaN pathway, and myocardial hypertrophy may further contribute to SCD. Therefore SOCS-1 participates in the pathogenesis of cardiac diseases by interacting with the JAK/STAT signaling pathway and its regulation of the cytokines. According to the study of Ottani A et al. [20], the JAK/STAT signaling pathway fulfills a crucial role in the early stage and second window of protection by ischemic preconditioning of myocytes. The upregulation of SOCS-1 may inhibit the expression of various cytokines in the myocytes, thus contribute to myocardial hypertrophy via the JAK/STAT signaling pathway and MAPK signaling pathway. Moreover, by inhibiting the JAK/STAT signaling pathway, upregulation of SOCS-1 can weaken the protective effect against the ischemic injury of the myocytes, which further increases the risk of SCD.

SOCS-1 can regulate the expression of cytokines expressed by the myocytes and participate in the pathogenesis of myocardial hypertrophy via the JAK/STAT signaling pathway and MAPK signaling pathway. Its upregulation is one of the major risk factors of SCD.

Conclusions

Abnormal expression of SOCS-1 and CX43 in cardiac tissues is closely related to SCD. Downregulation of CX43 will aggravate myocyte injury, thus increasing the risk of SCD. In contrast, upregulation of SOCS-1 will inhibit the expression of cytokines and further aggravate myocyte injury via the JAK/STAT signaling pathway and MAPK signaling pathway, thus contributing to SCD. Therefore, downregulation of CX43 and upregulation of SOCS-1 are the risk factors of SCD.

Disclosure of conflict of interest

None.

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References

[12] Wang K, Xu BC, Duan HY, Zhang, Hu FS. Late cardioprotection of exercise preconditioning against exhaustive exercise-induced myocardial injury by up-regulation of connexin 43 ex-


