Expressions of SOCS-1 and SOCS-3 in the myocardium of patients with sudden cardiac death

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BACKGROUND: As the regulators of cytokines, suppressors of cytokine signaling (SOCS) play an important role in the inflammation reaction. Some studies found that SOCS-1 and SOCS-3 were involved in the pathogenesis of some inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease. But the expressions of SOCS in coronary heart disease have not yet been reported. This study aimed to investigate the expression and clinical significance of SOCS-1 and SOCS-3 in the myocardium of patients with sudden cardiac death (SCD).

METHODS: Myocardial autopsy specimens were collected from 24 patients at the Forensic Medicine Department of Sun Yat-Sen University, Guangzhou, China between 2005 and 2006. Of them, 9 patients had autopsy findings consistent with coronary atherosclerosis (non-myocardial infarction) leading to SCD (non-MI group), 7 died of acute myocardial infarction (MI group), and 8 died from traffic accidents and trauma (control group). The expressions of SOCS-1 mRNA and SOCS-3 mRNA in the myocardium of the non-MI, MI and control groups were detected using RT-PCR. The levels of SOCS-1 and SOCS-3 proteins were detected using immunohistochemistry. Statistical analyses were performed using SPSS version 13.0 software and the data were analyzed by ANOVA.

RESULTS: The expressions of SOCS-1 mRNA and SOCS-3 mRNA in the non-MI and MI groups were significantly higher than those in the control group [(0.788±0.101), (0.741±0.111) vs. (0.436±0.044), (0.841±0.092), (0.776±0.070) vs. (0.454±0.076), (P<0.01)] respectively. The antibody-positive cells of SOCS-1 protein in the myocardium of the non-MI and MI groups were significantly higher than those in the myocardium of the control group [(320.00±48.48), (347.14±70.88) vs. (42.50±10.35), (P<0.01)] respectively. The antibody-positive cells of SOCS-3 protein in the myocardium of the non-MI and MI groups were significantly higher than those in the myocardium of the control group [(381.11±59.25) vs. (40.00±10.69), (P<0.01)] and [(332.86±111.91) vs. (40.00±10.69), (P=0.001)].

CONCLUSION: The expressions of SOCS-1 and SOCS-3 in the myocardium of patients with SCD from coronary heart disease are significantly increased and contribute to the pathogenesis of SCD.

KEY WORDS: Sudden cardiac death; Myocardial infarction; Suppressor of cytokine signaling-1; Suppressor of cytokine signaling-3

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INTRODUCTION
With the increasing living standards of the people in recent years, cardiovascular diseases have become common diseases in China. Sudden cardiac death (SCD) is one of the important issues in the prevention and control of cardiovascular diseases. It has been reported that 75%-80% of the patients died of SCD caused by coronary heart disease, and that diffuse coronary atherosclerosis developed in these patients. Inflammation reaction runs through the occurrence and development...
of atherosclerosis, and cytokines are involved in the inflammation reaction. As the regulators of cytokines, suppressors of cytokine signaling (SOCS) also play an important role in the inflammation reaction. Studies found that SOCS-1, SOCS-3 are involved in the pathogenesis of some inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. But the expressions of SOCS in coronary heart disease have not yet been reported. This study was undertaken to detect the possible relationship between SOCS-1, SOCS-3 and SCD caused by coronary heart disease.

METHODS

Patients
Myocardial autopsy specimens were collected from 24 patients at the Forensic Medicine Department of Sun Yat-Sen University between 2005 and 2006. Of them, 9 patients had autopsy findings consistent with coronary atherosclerosis (non-myocardial infarction) leading to SCD (non-MI group), 7 died of acute myocardial infarction (MI group), and 8 died from traffic accidents and trauma (control group). Each patient had detailed clinical information and autopsy records. The patients in the non-MI group were 25 to 67 years old (mean 45.11±14.66 years), the patients in the MI group were 32 to 60 years old (mean 45.29±12.15 years), and the patients in the control group were 26 to 52 years old (mean 37.75±8.26 years).

Main reagents
The following reagents were used: Trizol 100 ml (Invitrogen Company, USA), rabbit anti-human SOCS-1 polyclonal antibody (ab3691, ABCAM Company, UK), rabbit anti-human SOCS-3 polyclonal antibody (ab16030, ABCAM Company, UK), and Ultra Sensitive SP-Kit (Fuzhou, China).

Detection of SOCS-1 mRNA and SOCS-3 mRNA
After RNA extraction, RT reaction, cDNA synthesis and PCR amplification, 8 μl PCR amplification product was collected for electrophoresis with 2% agarose gel (70W, 45 minutes), and then was stained with 5 μg/μl ethidium bromide (EB) for 10 minutes. The A value of electrophoresis stripe was determined with the gel scanning system. The A values of SOCS-1, SOCS-3 and the reference stripe were calculated respectively, and served as the relative levels of mRNA.

Immunohistochemistry of SOCS-1 and SOCS-3
A piece of the left ventricular myocardium was taken, and was cut into 5 μm thick sections after being fixed and paraffin embedded with 10% formalin. The sections were subjected to routine HE staining of the myocardium and immunohistochemistry staining of SOCS-1 and SOCS-3. The immunohistochemical staining followed the instructions of Ultra Sensitive SP-Kit. The brown granules in cytoplasm served as positive staining. The diluted concentration of the first antibody was 1:100 for SOCS-1 and 1:200 for SOCS-3. PBS instead of the first antibody served as negative control. With the automatic image analysis system (Germany KONTRON IBAS 2.5), 10 regions were randomly selected in high power field in each section. Totally 100 cells were counted in each region, and were added with the antibody-positive cell number, that was, for positive number of every thousand myocardial cells, expressed by permillage (‰).

Statistical analysis
All data were expressed by mean ± standard deviation. The data of each group were subjected to analysis of variance (ANOVA) test. Statistical analyses were performed using SPSS version 13.0 software. A P value less than 0.05 was considered statistically significant.

RESULTS

Comparison of SOCS-1 mRNA and SOCS-3 mRNA
The expressions of SOCS-1 mRNA and SOCS-3 mRNA in the non-MI and MI groups were significantly higher than those in the control group (P<0.01). The comparison of SOCS-1 mRNA and SOCS-3 mRNA between the non-MI and MI groups was not significant (P>0.05) (Table 1, Figure 1).

Protein expressions of SOCS-1 and SOCS-3
The antibody-positive cell number of SOCS-1 and SOCS-3 proteins per thousand myocardial cells, expressed permillage (%) was counted. The antibody-positive cells of SOCS-1 and SOCS-3 proteins in the myocardium of the non-MI and MI groups were significantly higher than those in the myocardium of the control group (P<0.01). The comparison of SOCS-1 and SOCS-3 proteins between the non-MI and MI groups was not significant (P>0.05) (Table 2, Figures 2, 3).
DISCUSSION
Based on the myocardial autopsy specimens, we examined the expressions of SOCS-1 mRNA and SOCS-3 mRNA using RT-PCR, simultaneously examined the expressions of SOCS-1 and SOCS-3 proteins using immunohistochemistry, counted the antibody-positive cell number, and compared with the control group. The results of the study showed that the expressions of SOCS-1 mRNA and SOCS-3 mRNA and the antibody-positive cell number of SOCS-1 and SOCS-3 proteins in the non-MI and MI groups were significantly higher than those in the control group.

Recent studies on SOCS and heart disease revealed that the expressions of SOCS-3 in myocardial cells may contribute to cardiac hypertrophy.\(^{[4,5]}\) SOCS-1 in myocardial cells has not been fully studied, but it is recognized to weaken the signal of lipopolysaccharide (LPS) in macrophages and tumor necrosis factor (TNF).\(^{[6,8]}\) SOCS

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**Table 1.** Comparison of SOCS-1 mRNA and SOCS-3 mRNA between the control group, non-MI group and MI group (mean±SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>SOCS-1 mRNA</th>
<th>SOCS-3 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>0.436±0.044</td>
<td>0.454±0.076</td>
</tr>
<tr>
<td>Non-MI</td>
<td>9</td>
<td>0.788±0.101</td>
<td>0.841±0.092</td>
</tr>
<tr>
<td>MI</td>
<td>7</td>
<td>0.741±0.111</td>
<td>0.776±0.070</td>
</tr>
</tbody>
</table>

The expressions of SOCS-1 mRNA and SOCS-3 mRNA in each group were significantly different (\(F=36.822, P<0.01, F=53.623, P<0.01\)). Compared with the control group, \(^*P<0.01\).

**Table 2.** Expressions of SOCS-1 and SOCS-3 between the control, non-MI and MI groups (mean±SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>SOCS-1 (%)</th>
<th>SOCS-3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>42.50±10.35</td>
<td>40.00±10.69</td>
</tr>
<tr>
<td>Non-MI</td>
<td>9</td>
<td>320.00±48.48</td>
<td>381.1±59.25</td>
</tr>
<tr>
<td>MI</td>
<td>7</td>
<td>347.14±70.88</td>
<td>332.86±111.91</td>
</tr>
</tbody>
</table>

The expressions of SOCS-1 and SOCS-3 proteins in each group were significantly different (Welch analysis \(F=185.526, P<0.01; F=154.858, P<0.01\)). Compared with the control group, \(^*P<0.01\).
is the important negative regulatory protein of the JAK/STAT pathway and inflammation reaction. It is possibly involved in the pathogenesis of heart disease, with change in the JAK/STAT pathway and inflammation reaction in the heart.

Hypoxia, inflammation and tension load can activate the JAK-STAT pathway. Acute myocardial infarction is a response of the complex pathological process associated with an acute shortage of oxygen supply and secondary inflammation and mechanical stress. Thus, the activation of the JAK-STAT pathway in acute myocardial infarction is worth studying. Negoro et al.[9] found that the region of myocardial infarction and marginal zone had STAT3 phosphorylation. Only a small amount of apoptotic myocardial cells were found in the marginal zone of myocardial infarction. JAK2 inhibitor AG490 significantly inhibited STAT3 phosphorylation, increased myocardial caspase-3 activity and expression of Bax, while apoptotic myocardial cells increased significantly in the marginal zone of myocardial infarction, indicating that the activation of JAK-STAT could prevent myocardial cell apoptosis after myocardial infarction. The existing evidence indicated that STAT1 and STAT3 activation had an opposite effect on the heart. STAT3 activation inhibited the apoptosis, played a role in myocardial protection, and STAT1 activation induced apoptosis.[10,11] Stephanou et al.[12] reported that the ischemia-caused myocardial cells apoptosis, accompanied by increased expression and transcriptional activity of STAT1, antisense STAT1 vector inhibited ischemia-induced myocardial cells apoptosis, but overexpression of STAT1 increased ischemia-induced apoptosis. Immunofluorescence staining showed that apoptotic cells were STAT1 positive, and proved powerfully that ischemia-reperfusion induced myocardial cells apoptosis by activation of the STAT1 signaling pathway.

There is a network construction among STAT1, STAT3, SOCS-1 and SOCS-3. STAT1 induces apoptosis, inhibits cell growth, activates macrophages, dendritic cells and other antigen presenting cells, promotes the release of inflammatory mediators and their signal transduction, and amplifies the inflammatory process. STAT3 has an effect on inhibition of apoptosis, promotion of cell growth and repair of injured tissues, and to some extent limits the inflammatory process of body injury. SOCS-1 is mainly induced by STAT1. SOCS-1 inhibits apoptosis induced by STAT1 and tissue damage, reduces inflammation damage sensitivity of organization to protect the organization to avoid the inflammatory injury. SOCS-3 is mainly induced by STAT3. It has an impact on the anti-apoptosis of STAT3 and the process of tissue repair. Myocardial ischemia activates STAT1 and STAT3, induces SOCS-1 and SOCS-3. This may be the reason that the expressions of myocardial SOCS-1 and SOCS-3 elevated after SCD caused by coronary heart disease.

Inflammatory reaction exists during the occurrence and development of atherosclerotic lesions.[13] Coronary artery inflammation leads to endothelial dysfunction, plaque formation and progress, ultimately leads to plaque rupture. Thus the plaque lipid-rich core exposes to circulating blood, leading to platelet adhesion, aggregation, thrombus formation on the plaque surface, further leading to coronary occlusion, myocardial necrosis, and acute coronary syndrome.[14,15] In this study, the autopsy result of the non-MI group was consistent with coronary atherosclerosis leading to sudden death. Coronary atherosclerosis, plaque rupture or thrombosis, and myocardial necrosis were found by autopsy examination of the MI group. Therefore, SOCS may have an effect on atherosclerotic lesions and participate in the pathogenesis of SCD caused by coronary heart disease. This is why there were no significant differences in the expressions of SOCS-1 and SOCS-3 between the two groups in the present study.

The present study showed that SOCS-1 and SOCS-3 may participate in the pathogenesis of SCD. Because of the limited sample of the study, limitations were obvious in study design and methods. The relations and underlying mechanism of SOCS-1, SOCS-3, coronary heart disease, and SCD need further study.

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