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Original Article

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

Table 1. Primer design

Gene	Primer sequence (5'-3')	Length (bp)
SOCS-1	F: AGCCTTTCTCCGGCCCTA	243
	R: GTTCAGCCTCAGTGGACACA	
CX43	F: GCAGCCATTGGCGTTGATAG	380
	R: ATCGCCAAGGTCTGTTCTGG	
β-actin	F: AAGTACTCCGTGTGGATCGG	615
	R: TCAAGTTGGGGGACAAAAAG	

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

Groups	N/case	CX43	SOCS-1
SCD patients	21	0.395+0.014	0.892+0.103
Non-SCD patients	21	0.823+0.021	0.429+0.037
t	---	19.336	18.267
P	---	<0.05	<0.05

Pufei Biotech Co., Ltd.), and immunohistochemical staining kit (Shanghai Jiyan Biotech Co., Ltd.).

RT-PCR detection of mRNA expression of CX43 and SOCS-1 in the cardiac tissues

Total RNA was extracted from the cardiac tissues using the tissue RNA extraction kit. RNA concentration was determined. 1000 ng of cDNA was generated by using the reverse transcription kit (20 μl). Then 20 μl MilliQ water was added and RT-PCR was performed using cDNA as the template. The primers were designed for SOCS-1 and CX43 according to the sequences downloaded from NCBI (**Table 1**). After 1.5% agarose gel electrophoresis (120 V, 15 min) was performed using 10 μl PCR product, the gel was scanned by Bio-Rad imaging system, and the gel images were analyzed using Quantity One. The relative expression of the target genes was determined with β-actin as an internal reference marker.

Immunohistochemical detection of CX43 and SOCS-1 protein expressions in cardiac tissues: The tissues were incubated with primary antibodies and subjected to immunohistochemical staining using the immunohistochemistry kit. For negative control, PBS was added instead. Ten fields of view were selected randomly for each slice (400 × magnification), and the positive area (S value) and mean optical densities (OD value) of CX43 and SOCS-1 were determined.

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

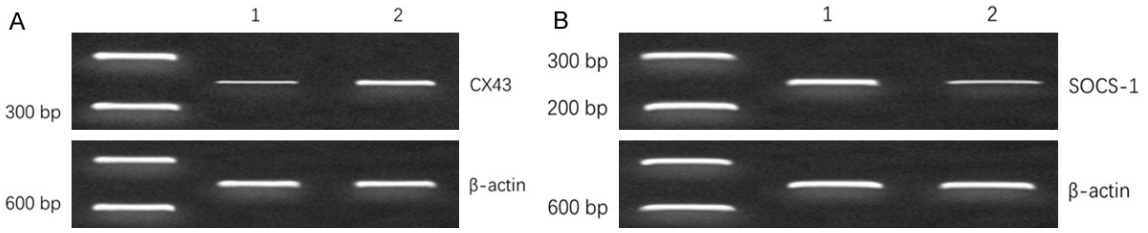

Figure 1. MRNA expression of CX43 and SOCS-1 and in the two groups. 1 stands non-SCD patients group; 2 stands SCD patients group.

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

Groups	N/case	CX43		SOCS-1	
		S	OD	S	OD
SCD patients	21	1.538+0.328	0.42+0.003	2.231+0.515	1.129+0.002
Non-SCD patients	21	2.402+0.928	1.421+0.007	1.369+0.257	0.201+0.005
t		132.024	25.127	105.371	15.664
P		<0.05	<0.05	<0.05	<0.05

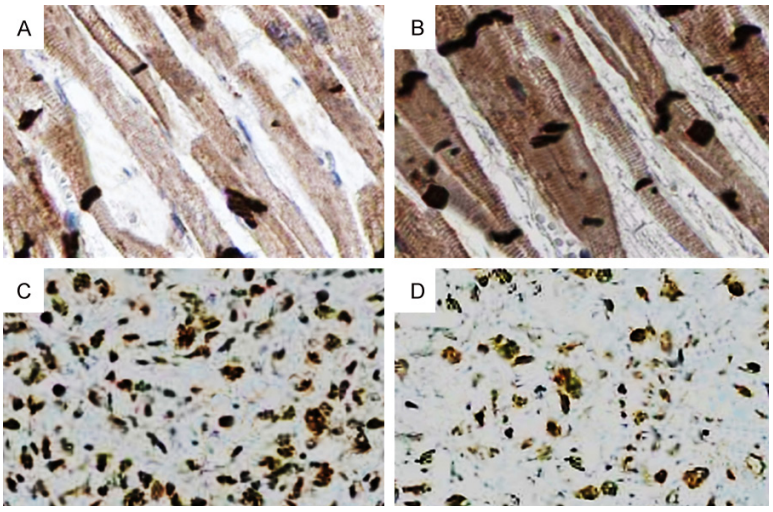

Figure 2. Immunohistochemical staining of CX43 and SOCS-1 in the cardiac tissues (400 ×). A: CX43 expression in the cardiac tissues of the SCD patients; B: CX43 expression in the cardiac tissues of the non-SCD patients; C: SOCS-1 expression in the cardiac tissues of the SCD patients; D: SOCS-1 expression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

injury. Wang *et al.* [12] showed that myocyte injury was associated with a downregulation of CX43 and a change in the distribution pattern of CX43 in the myocytes. CX43 is an important molecule associated with myocyte injury. Saez JC *et al.* [13] demonstrated the molecular mechanism involving CX43 in the mediation of myocyte injury. As CX43 is downregulated, there will be a considerable influx of Na^+ and Ca^{2+} into the myocytes, leading to a high loss of intracellular metabolites and hence ischemic injury caused by ATP leakage. We deduce that 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

[1] Kuch M, Janiszewski M, Mamcarz A, Cudnoch-Jedrzejewska A, Dłuzniewski M. Major adverse cardiac event predictors in survivors of myocardial infarction with asymptomatic left ventricular dysfunction or chronic heart failure. Med Sci Monit 2009; 15: PH40-48.

[2] Fan G, Zhang L. [Sudden cardiac death: progress in epidemiological research]. Zhonghua Liu Xing Bing Xue Za Zhi 2015; 36: 87-89.

[3] John RM, Tedrow UB, Koplan BA, Albert CM, Epstein LM, Sweeney MO, Miller AL, Michaud GF, Stevenson WG. Ventricular arrhythmias and sudden cardiac death. Lancet 2012; 380: 1520-1529.

[4] Lam CS, Anand I, Zhang S, Shimizu W, Narasimhan C, Park SW, Yu CM, Ngarmukos T, Omar R, Reyes EB, Siswanto B, Ling LH, Richards AM. Asian sudden cardiac death in heart failure (ASIAN-HF) registry. Eur J Heart Fail 2013; 15: 928-936.

[5] Tagami-Nagata N, Serada S, Fujimoto M, Tanemura A, Nakatsuka R, Ohkawara T, Murota H, Kishimoto T, Katayama I, Naka T. Suppressor of cytokine signalling-1 induces significant preclinical antitumor effect in malignant melanoma cells. Exp Dermatol 2015; 24: 864-871.

[6] Forster T, Rausch V, Zhang Y, Isayev O, Heilmann K, Schoensiegel F, Liu L, Nessling M, Richter K, Labsch S, Nwaeburu CC, Mattern J, Gladkich J, Giese N, Werner J, Schemmer P, Gross W, Gebhard MM, Gerhauser C, Schaefer M, Herr I. Sulforaphane counteracts aggressiveness of pancreatic cancer driven by dysregulated Cx43-mediated gap junctional intercellular communication. Oncotarget 2014; 5: 1621-1634.

[7] Solan JL, Lampe PD. Specific Cx43 phosphorylation events regulate gap junction turnover in vivo. FEBS Lett 2014; 588: 1423-1429.

[8] de Wit C, Griffith TM. Connexins and gap junctions in the EDHF phenomenon and conducted vasomotor responses. Pflugers Arch 2010; 459: 897-914.

[9] Severs NJ, Bruce AF, Dupont E, Rothery S. Remodelling of gap junctions and connexin expression in diseased myocardium. Cardiovasc Res 2008; 80: 9-19.

[10] Tribulova N, Seki S, Radosinska J, Kaplan P, Babusikova E, Knezl V, Mochizuki S. Myocardial Ca2+ handling and cell-to-cell coupling, key factors in prevention of sudden cardiac death. Can J Physiol Pharmacol 2009; 87: 1120-1129.

[11] Rhett JM, Jourdan J, Gourdie RG. Connexin 43 connexon to gap junction transition is regulated by zonula occludens-1. Mol Biol Cell 2011; 22: 1516-1528.

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

pression in rat hearts. Asian Pac J Trop Med 2015; 8: 658-663.
[13] Sáez JC, Schalper KA, Retamal MA, Orellana JA, Shoji KF, Bennett MV. Cell membrane permeabilization via connexin hemichannels in living and dying cells. Exp Cell Res 2010; 316: 2377-2389.
[14] Feng X, Tang H, Leng J, Jiang Q. Suppressors of cytokine signaling (SOCS) and type 2 diabetes. Mol Biol Rep 2014; 41: 2265-2274.
[15] Galic S, Sachithanandan N, Kay TW, Steinberg GR. Suppressor of cytokine signalling (SOCS) proteins as guardians of inflammatory responses critical for regulating insulin sensitivity. Biochem J 2014; 461: 177-188.
[16] Čokić VP, Mitrović-Ajtić O, Beleslin-Čokić BB, Marković D, Buač M, Diklić M, Kraguljac-Kurtović N, Damjanović S, Milenković P, Gotić M, Raj PK. Proinflammatory cytokine IL-6 and JAK-STAT signaling pathway in myeloproliferative neoplasms. Mediators Inflamm 2015; 2015: 453020.
[17] Liu J, Shen Q, Wu Y. Simvastatin prevents cardiac hypertrophy in vitro and in vivo via JAK/STAT pathway. Life Sci 2008; 82: 991-996.
[18] Streicher JM, Ren S, Herschman H, Wang Y. MAPK-activated protein kinase-2 in cardiac hypertrophy and cyclooxygenase-2 regulation in heart. Circ Res 2010; 106: 1434-1443.
[19] Jeong D, Kim JM, Cha H, Oh JG, Park J, Yun SH, Ju ES, Jeon ES, Hajjar RJ, Park WJ. PICOT attenuates cardiac hypertrophy by disrupting calcineurin-NFAT signaling. Circ Res 2008; 102: 711-719.
[20] Ottani A, Galantucci M, Ardimento E, Neri L, Canalini F, Calevro A, Zaffe D, Novellino E, Grieco P, Giuliani D, Guarini S. Modulation of the JAK/ERK/STAT signaling in melanocortin-induced inhibition of local and systemic responses to myocardial ischemia/reperfusion. Pharmacol Res 2013; 72: 1-8.