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Down-regulation of Connexin 43 mRNA in Mouse Hearts after Myocardial Infarction

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Abstract: Gap junction remodeling have been reported in various types of heart disease. Information available for the gap junction remodeling after myocardial infarction is still limited. In the present study, we investigated mRNA expression of connexin 43 (Cx43), major protein of gap junction channels in compensated hypertrophied interventricular septum (IVS) after myocardial infarction (MI) in mice by using a real time quantitative PCR expression. Cx43 mRNA expression in the interventricular septum of MI mouse was significantly decreased by 2 fold (813.7±252.6 molecules / 10^5 GAPDH molecules) compared to non-operated control (1628.9±180.9) and sham-operated mice (2051.9±169.8). There was no significant difference in Cx43 mRNA between control and sham groups. Down-regulation of Cx43 mRNA in compensated hypertrophied myocardium could be involved in the arrythmogenic substrate in the heart after MI.

Key words: gap junction, connexin43, mouse, hypertrophy, myocardial infarction

Cardiac arrhythmia is a common and often lethal manifestation of many forms of heart disease including hypertrophy, heart disease, and myocardial infarction (MI). Gap junction remodeling has been postulated to contribute to produce arrythmogenic substrate in such diseased hearts. (Toon et al., 2001). Connexin 43 (Cx43) are major subtypes of gap junction channel proteins in mammalian working myocardium (Kanno et al., 2001). In previous reports, Cx43 expressions are decreased in border zone of myocardial infarction in human and rat (Matsushita et al., 1999). However, transcriptional changes of Cx43 in compensated hypertrophied muscle after MI remain to be studied. In the present study, we analyzed quantitatively the expression of Cx43 mRNA in the interventricular septum (IVS) of mouse heart after MI.

Materials and Methods

1. Mouse MI model

Female ICR mice, weighting 28–33 g (9–11 weeks old), were randomly divided to three groups (MI, sham operation, and non-operation). MI was induced by ligation of the left coronary artery. Mice were anesthetized with pentobarbital (50 mg/kg, i.p.). The thoracotomy was performed under artificial respiration. After the pericardial sac was opened and the heart was exteriorized through the intercostal space, the left anterior descending artery was ligated using 8–0 prolene with an atraumatic needle (Ethicon). Then, the thorax was closed and the skin was sutured with 5–0 prolene. Sham operated animals were used as references.

2. Total RNA extraction and reverse transcription

At 5–7 weeks after the operation, the interventricular septum (IVS) in MI mice showed the macroscopic hypertrophy. Total RNA was extracted from the hypertrophied septum using the Acid Guanidinium-Phenol-Chloroform (AGPC) method. After treatment with Dnase I, single-stranded cDNA was synthesized by an oligo d(T) primer and SuperScript II Rnase H-reverse transcriptase (Invitrogen BRL).

3. Quantification of Cx43 mRNA expression

The real-time PCR assay was performed to estimate the expression of Cx43 mRNA (ABI Prism 7700). The primers and probes were designed as shown in Table 1. PCR product was subcloned using TA cloning and sequenced. cDNA molecules of PCR product (10^3–10^7 molecules) were amplified for the determination of standard curve. GAPDH gene was used as an endogenous control, because there was no difference of GAPDH expressions among control, sham and MI groups.

4. Data analysis

Data are presented as mean±SEM. Statistical analysis of data was performed using non-paired t test. Differences were considered significant at $p<0.01$.

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Table 1 Sequence of PCR primers and sequence specific probes for connexin 43 and GAPDH.

Target sequence	Accesion No.	Primer	Sequence (5'→3')	Position	Amplicon length (bp)
connexin 43	X62836	sense	aaaatcgaatggggcaggc	1105–1123	77
		probe	ctctgcgctgtaattcgcccagttttgc	1103–1076	
		antisense	tgcttgttgtaattgcggca	1047–1066	
GAPDH	M32599	sense	cttcaccaccatggagaaggc	343–363	238
		probe	cctggccaaggtcatccatgacaacttt	517–544	
		antisense	ctcatgaccacagtccatgcc	560–580	

Results and Discussion

Cx43 mRNA levels in the myocardium were quantified by real-time PCR at 5–7 weeks after surgical operation. Cx43 mRNA expression in IVS of MI mouse significantly decreased by 2 fold (813.7±252.6 molecules / 10^5 GAPDH molecules, n=7) compared to non-operated control (1628.9±180.9 molecules / 10^5 GAPDH molecules, n=6) and sham-operated mice (2051.9±169.8 molecules / 10^5 GAPDH molecules, n=6) (Figure 1). There was no significant difference of Cx43 between control and sham groups.

Many reports have been published demonstrating substantial changes in gap junction distribution, density, and properties in a variety of structural heart disease including hypertention, heart failure, myocardial infarction and chronic atrial fibrillation. The gap junction remodeling is supposed to produce arrythmogenic substrate by altering conduction properties of the heart predisposing reentry of excitation (Habo et al., 2000). In border zone of myocardial infarction and hypertrophic cardiomyopathy in human and animal heart, the decrease of Cx43 protein level, the transverse conduction slowing, and the change of anisotropic ratio have been reported (Pertes et al., 1993; Matsushita et al., 1999; Uzzaman et al., 2000; Kanno et al., 2003; Yao et al., 2003). Our results are concordant with this previous report and suggest that those changes are the result of gene transcription of Cx43. We did not compare Cx43 mRNA level between border zone of myocardial infarction and compensated hypertrophied IVS, and did not perform analysis the distribution pattern using immunohistochemistry. Further experimental studies will be required to clarify the regional difference of Cx43 transcription.

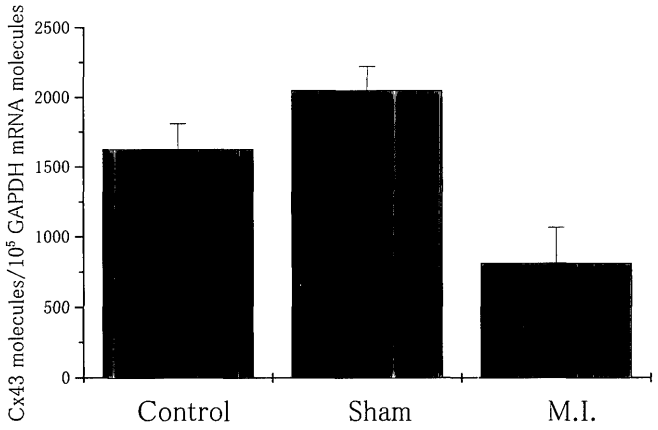

Fig. 1 Quantification of connexin 43 mRNA expression assessed by real-time PCR in the interventricular septum of mice at 5–7 weeks after myocardial infarction (MI), sham operation (sham) and non-operated control mice (control).

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