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Study on the spatial distribution pattern of Cx40 gap junctions in the atria of patients with coronary heart disease

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Abstract

Background: *The aim of this study was to evaluate the association between atrial fibrillation and atrial dilation and the spatial distribution pattern of connexin 40 in the atria of patients with coronary heart disease.*

Methods: *Twenty-six patients with coronary heart disease undergoing cardiac surgery for coronary artery bypass graft were investigated and were divided into three groups according to the left atrial size and rhythm, atrial fibrillation and left atrial dilatation (AF+AD), sinus rhythm and left atrial dilation (SR+AD) and sinus rhythm as control group SR. The spatial distribution patterns of Cx40 were evaluated using confocal laser scanning microscopy assay.*

Results: *No significant differences were observed in the size and density of Cx40 gap junction in the right atrium among all three groups ($p > 0.05$). Compared with the control group, the size of Cx40 disk area in termination links and in lateral abutment in left atrium was markedly larger in AF+AD group and SR+AD group than those of the controls ($p < 0.01$). A comparison of size and density of Cx40 gap junction in the left atrium in the AF+AD group and SR+AD group did not show significant differences.*

Conclusions: *The present study has shown altered gap junction distribution in coronary heart disease resulting from atrial dilation and atrial fibrillation. A decrease in the size and density of Cx40 gap junction was observed in patients with atrial dilation, which could be an important factor in the initiation and maintenance of atrial fibrillation.* (Cardiol J 2008; 15: 50–56)

Key words: coronary heart disease, atrial dilation, atrial fibrillation, connexin 40

Introduction

Atrial fibrillation (AF) frequently occurs in patients with atrial enlargement, which results from a variety of mechanical stresses to the atrium. Atrial dilatation (AD) is considered to be one of the most important factors in the initiation and the perpetuation of AF. This may be attributed, in part, to an increase in excitable media, which facilitates the coexistence of multiple reentrant circuits, and, in part, to altered electrophysiological properties of atrial muscle [1]. In the heart, gap junctions provide the pathways for intercellular current flow, enabling coordinated action potential propagation. Gap-junctional channels are constructed from connexins (Cxs), a multigene family of conserved proteins. In the mammalian heart, Cx40 is present specifically in the atrium and in the specialized conducting system and is important for atrial arrhythmia (including AF) [2, 3]. In the present study,

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we investigated gap junction remodelling in atrial tissues from coronary artery disease patients with or without AF and appreciable left AD. The distribution and the expression of Cx40 proteins were analyzed with the aid of antibody labelling and confocal laser scanning microscopy.

Methods

Study population and tissue collection

Data on studied patients, tissue sampling characteristics and specimens are summarized in Table 1. Atrial appendages were obtained from 26 patients with coronary heart disease undergoing cardiac surgery for coronary artery bypass graft (CABG) and were divided into three groups according to the left atrial size and rhythm: six patients with atrial fibrillation and left atrial dilatation (AF+AD), eight patients with sinus rhythm (SR) and left atrial dilatation (SR+AD) and 12 patients with sinus rhythm as a control group (SR). In each case, one tissue sample was obtained from the left atrial (LA) appendage. The spatial distribution pattern of Cx40 was detected using confocal laser scanning microscopy assay. The left atrial diameter (LAD) measured by echocardiography was > 40 mm. No history of AF or other valvular or nonvalvular cardiopathy was found. There were no significant differences between the three groups in terms of age, diabetes mellitus or left ventricular ejection fraction. The tissue samples (10–18 mm in long axis, 6–12 mm in short axis) obtained either from the right or LA appendage were immediately frozen to prepare cryosections of 4–5 μm thickness, and then fixed with 2% paraformaldehyde in phosphate buffer saline for subsequent immunohistochemistry. Two to four immunolabelled sections from each sample were analyzed. The Institutional Ethics

Table 1. Clinical profiles of patients

Patient No.	Sex	Age (years)	Underlying disease	Rhythm	LAD/RAD [mm]	EF (%)	Sampling RAA/LAA
1	M	64	TVD	SR	36/31	66	RAA
2	M	58	OMI, DM	SR	29/30	64	RAA
3	F	60	OMI	SR	37/29	68	RAA
4	M	71	TVD	AF	47/33	41	RAA, LAA
5	M	62	OMI, DM	SR	45/35	46	RAA, LAA
6	M	73	TVD, DM	SR	34/32	58	RAA
7	F	68	OMI, DM	AF	48/34	44	RAA, LAA
8	M	76	OMI, DM	SR	34/35	56	RAA, LAA
9	M	67	TVD, DM	AF	46/32	48	RAA, LAA
10	M	77	OMI, DM	SR	33/30	64	RAA
11	M	63	OMI DM	SR	51/34	51	RAA, LAA
12	F	71	OMI	SR	49/31	53	RAA, LAA
13	M	56	OMI	AF	45/33	49	RAA, LAA
14	M	59	TVD, DM	SR	32/30	67	RAA
15	M	65	TVD, DM	SR	36/31	61	RAA
16	M	68	TVD, DM	SR	45/34	47	RAA, LAA
17	M	61	OMI, DM	SR	48/35	52	RAA, LAA
18	F	68	TVD, DM	AF	50/32	38	RAA, LAA
19	F	74	OMI, DM	SR	37/33	55	RAA
20	M	78	TVD, DM	SR	49/34	48	RAA, LAA
21	M	67	OMI, DM	AF	46/36	53	RAA, LAA
22	M	77	TVD	SR	34/29	67	RAA
23	F	64	TVD, DM	SR	46/31	49	RAA, LAA
24	M	53	OMI, DM	SR	38/33	61	RAA
25	M	79	TVD	SR	34/32	62	RAA
26	M	64	OMI, DM	SR	46/34	51	RAA, LAA

AD — left atrial dilatation; EF — ejection fraction; LAD — left atrial diameter; RAD — right atrial diameter; SR — sinus rhythm; OMI — old myocardial infarction; TVD — triple-vessel disease; DM — diabetes mellitus; RAA — right atrial appendages; LAA — left atrial appendages; AF — atrial fibrillation

Committee approved the present study, and all patients gave written consent by signing a specifically designed consent form.

Immunohistochemistry

For immunodetection of gap junctions, a polyclonal rabbit anti-Cx40 antibody (London, UK) was used. After permeabilization (0.3% TritonX-100), quenching (0.1 mol/L lysine) and blocking (3% goat serum/5% bovine serum albumin), samples were incubated with the antibody for two hours at room temperature. Primary antibody-bound Cx40 and cadherin were visualized using fluorescent isothiocyanate-conjugated anti-mouse immunoglobulin (Ig)G and Texas Redconjugated anti-rabbit IgG. Samples processed without primary antibodies served as negative controls. The labelled samples were examined using a confocal microscope (Bio--Rad MRC 1024). Atrial cell size (width and length) was measured in single optical slices sectioned along the fibre orientation. Ten cells were measured in each sample and then averaged. In addition to single plane evaluation, optical section series were taken (0.5-μm interval). The area of the main intercalated disk (disk area) and the area of immunolabelled Cx40 gap junction at the intercalated disk (gap junction area) were estimated in projection images of the disk using NIH images. En-face discs viewed in transversely sectioned tissue were used because, in this view, the overlap of individual gap junctions is minimized. The $\times$63 objective with a detector pinhole of 90 mm gave an optical section thickness of 0.5 mm on theoretical grounds. A zoom factor of 2.96 with a 512 $\times$ 512-pixel image was selected, yielding a pixel size of 0.1 $\times$ 0.1 mm. This allowed proportionate representation of the smallest junctions (1 mm) detectable with this technique.

Statistical analysis

Data are expressed as mean $\pm$ SD. The difference between groups was analyzed by using ANOVA and non-parametric procedures wherever appropriate. Details of the procedure used are stated in each figure legend. All effects and interactions are tested at a 0.05 level of significance.

Results

The disk area and gap junction area in the intercalated disk seen en-face was estimated using the protocol reported previously. Averaged values of ten disks were obtained in each sample, and then the mean$\pm$SD was calculated for each of the 41 samples (26 RA and 15 LA) from the patients.

Table 2. Comparing the disk area in termination links in LA and RA tissues with three groups [μm^2]

Group	LA samples	RA samples	LA	RA
SR	1	12	98.6 ± 6.3*	102.4 ± 5.7*
SR+AD	8	8	211.3 ± 6.4**	103.5 ± 6.3*
AF+AD	6	6	206.3 ± 5.9**	101.7 ± 5.6*

*p > 0.05; **p < 0.001; RA — right atrial; LA — left atrial;
SR — sinus rhythm, AD — left atrial dilatation; AF — atrial fibrillation

Table 3. Comparing the disk area in lateral abutment in LA and RA tissues with three groups [μm^2]

Group	LA samples	RA samples	LA	RA
SR	1	12	15.6 ± 2.3	14.9 ± 1.7
SR+AD	8	8	19.3 ± 2.4	15.5 ± 2.1
AF+AD	6	6	18.7 ± 2.3	15.7 ± 2.6

*p > 0.05; **p < 0.001; RA — right atrial; LA — left atrial;
SR — sinus rhythm, AD — left atrial dilatation; AF — atrial fibrillation

Ten intercalated disks randomly selected from each sample were analyzed.

In RA tissues, there were no significant differences in the disk area and the gap junction area among the three patient groups. In LA tissues, the disk areas in AF+AD and SR+AD patients were significantly greater than those of the controls ($p < 0.001$). The Cx40 gap junction areas in the LA tissues of AF+AD patients and SR+AD patients were slightly, but significantly, larger than those of the controls. There was no significant difference in these parameters between AF+AD and SR+AD patients. In control patients, there were no significant differences between RA and LA tissues in the disk area and in the gap junction area. The gap junction areas in LA tissues from AF+AD patients were significantly larger than those in RA tissues from the same patients and from SR patients (Table 2, 3).

Figures 1 and 2 show the immunolabelled Cx40 gap junctions in single confocal optical slices sectioned along the fibre orientation. In both AF+AD, the LA tissues showed a significantly more intercalated disk area than RA tissues. All right atrial (RA) and LA tissues from control (SR) patients disks showed a normal Cx40-labelling pattern with small central gap junctions surrounded by larger labelling spots at the disk periphery. In LA tissues from AF+AD and SR +AD patients, the peripheral gap junctions were preserved, but there was a prominent loss of the central small gap junctions,

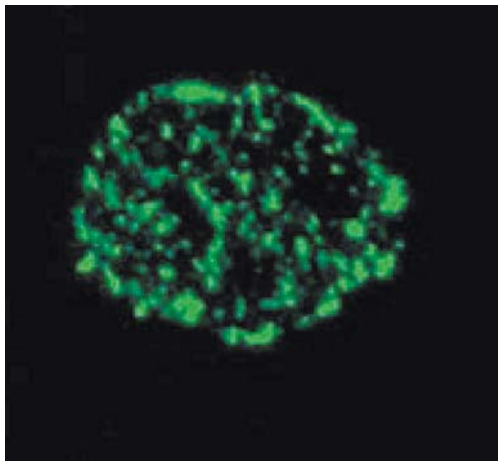

Figure 1. Right atrial (RA) myocardium from a control (SR, sinus rhythm) patient.

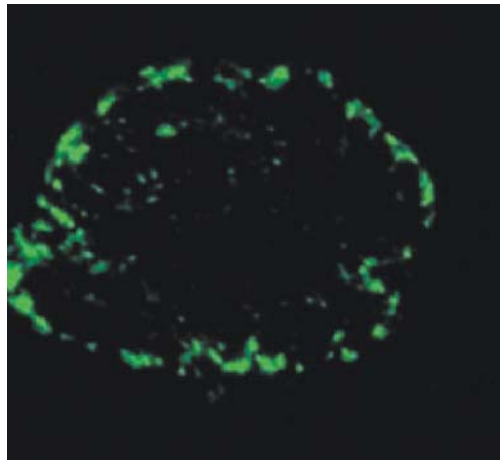

Figure 2. Left atrial (LA) myocardium from an atrial fibrillation and left atrial dilatation (AF+AD) patient (Bar = 10 μm), respectively. Immunostained connexin40 gap junctions at the intercalated disks viewed face-on. Projection images were constructed by superimposing optical slices at 0.5 μm intervals from atrial tissues sectioned across the myofibre. The LA tissues showed a significantly more intercalated disk area than right atrial tissues.

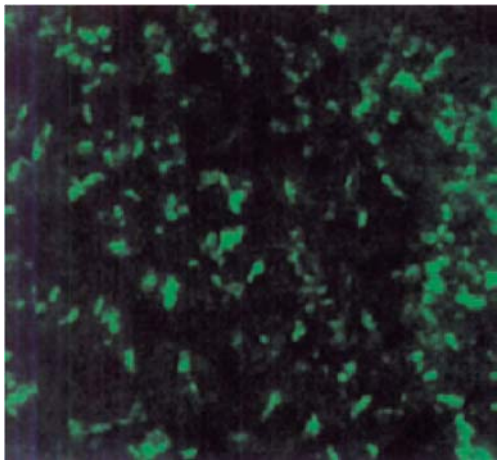

Figure 3. Confocal microscopy for connexin40 on atrial tissues sectioned along the myofibre.

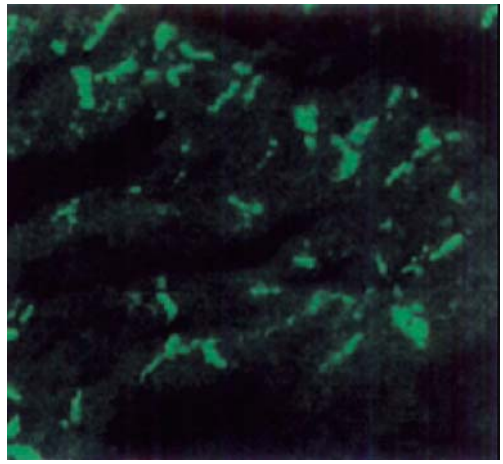

Figure 4. Connexin40 termini disk area and lateral abutment disk area in right atrial myocardium from a control patient with sinus rhythm

giving rise to a more empty appearance of the disk. A substantial increase in the disk size was also recognized.

In right atrial (RA) and LA (normal) tissues from control (SR) patients, Cx40-containing gap junctions were visualized as aggregates of bright punctate fluorescent domains not only at the cell termini, marking the position of intercalated disks, but also at the lateral abutment with neighbouring cells. Figures 3–5 show a normal Cx40-labelling pattern with small central gap junctions surrounded by larger labelling spots at the disk periphery. In Figure 6 the peripheral gap junctions are preserved, but there is a prominent loss of the central small gap junctions, giving rise to a more empty appearance of the disk. A substantial increase of the disk size was also recognized.

Discussion

In this study, we investigated the disorganization of gap junctions in the atrial muscles of AD or AF patients with coronary heart disease. The results revealed a remarkable loss of central small Cx40-gap junctions at the intercalated disk of AF

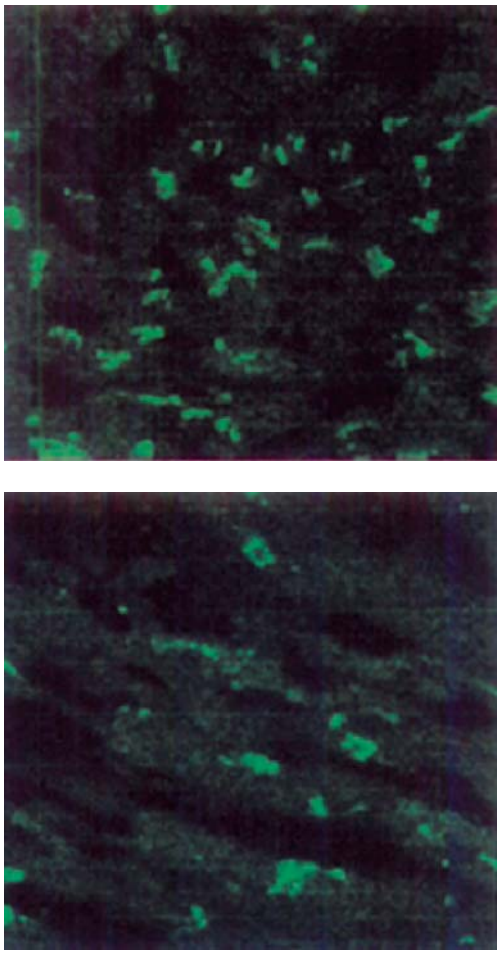

Figure 5. Left atrial (LA) myocardium from a patient with sinus rhythm (SR). Images are single optical slices (Bar = = 50 μm). Connexin40 was distributed not only in the intercalated disks but also in the lateral abutment of myocytes.

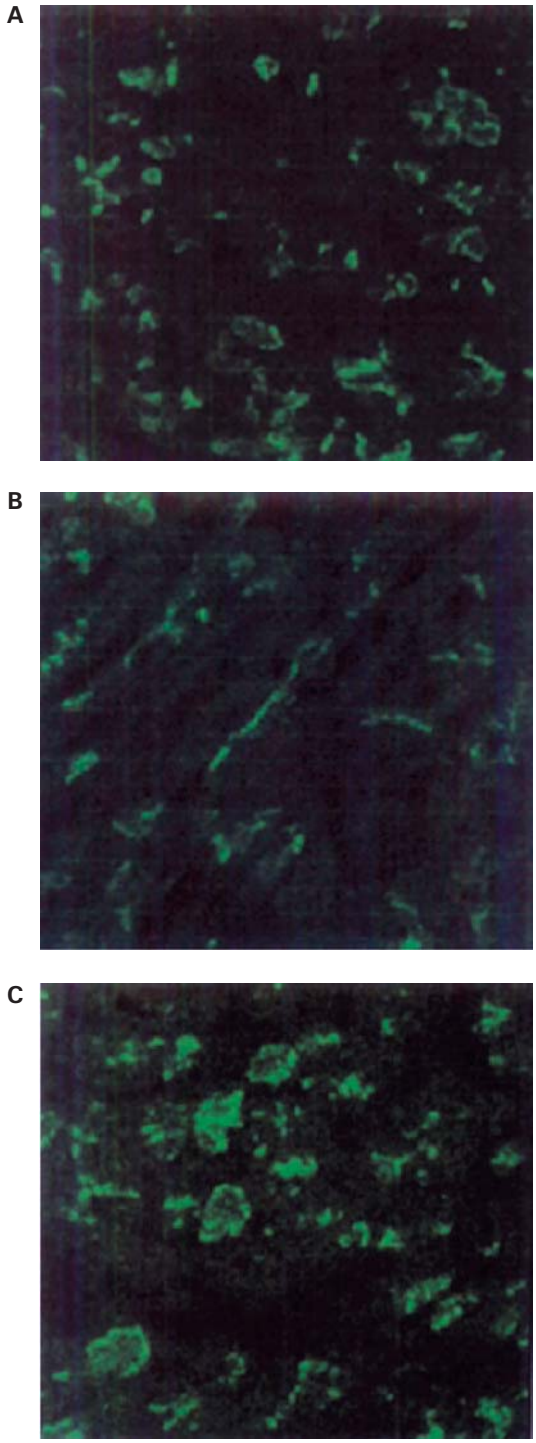

Figure 6. A. B. Connexin40 termini disk area and lateral abutment disk area in SR+AD. **C.** In AF+AD. Images are single optical slices (Bar = 50 μm). Connexin40 was distributed not only in the intercalated disks but also in the lateral abutment of myocytes. Left atrial (LA) myocardium from a patient with atrial fibrillation and left atrial dilatation (AF+AD) and sinus rhythm and left atrial dilatation (SR+AD). There was a prominent loss of the central small gap junctions, giving rise to a more empty appearance of the disk. A substantial increase of the disk size was also recognized.

patients. The reduction of Cx40 gap junctions within the intercalated disk was remarkable in the LA and occurred concomitantly with an enlargement of disk area, while the changes of both Cx40 distribution and disk area were negligible in the RA. Analogous changes in Cx40 and disk area were observed in the LA in patients with SR+AD. Although the number of samples is still small, especially in the group of AF+AD, the results taken altogether strongly suggest that the gap junction remodelling was brought about by AD and AF and the frequency of local electrical excitation.

A number of studies involving both human and experimental animals have documented changes in the cellular electrophysiology in AF. These include a shortening of action potential duration (APD), a shortening of refractory period (RP), a loss of rate

adaptation of APD and RP, and an increased dispersion of APD and RP in the atria [4–6]. This electrical remodelling, which is probably the result of altered gene expression of ion channels [7], might be responsible for the well-known fact that cardioversion has a much higher success rate when AF is of recent onset. The changes in APD and RP reach a steady state within a few days following induction of AF, whereas it often takes a few additional weeks for AF to become persistent. The study by Nao et al. [8] found a significant decrease in Cx40 mRNA/protein amounts. The discrepancy among the reports may be explained in part by the spatial heterogeneity of Cx40 in the atria. In the present study on human atrial tissue, the labelling intensity of Cx40 was inhomogeneous with regions of myocardium showing little staining adjacent to other areas that were labelled intensely. Kostin et al. [9] showed that there was a statistically larger area of Cx40 expression in RA tissues from AF patients than from patients in the SR group.

Other factors might also play an important role in the development of chronic AF [10, 11]. Recent experimental examinations suggest that changes of expression and distribution of gap junctions could be involved [12, 13]. In our findings, the total gap junction area per disk was slightly decreased in enlarged LA. This would best be compatible with decreasing propagation velocity. The increase in cell volume would be expected to increase with conduction velocity. In reality, however, the functional consequences might be more complex because of the well-documented remodelling of ion channels, including the downregulation of the fast Na channel, and co-operative interaction between individual connexons in gap junctions [14]. In linearly structured myocardium, a slowing of conduction would increase the likelihood of the occurrence of arrhythmias via a shortening of the wavelength of excitation [15]. However, a partial decrease of cell-to-cell coupling has been shown to render conduction more homogeneous and to reverse unidirectional block to bidirectional conduction in tissue characterized by a markedly discontinuous architecture [16, 17]. Therefore, the contribution of altered cell-to-cell coupling to arrhythmogenesis has to be judged using an integrated concept that takes all remodelled variables into account, such as tissue architecture, ion channels and cell size in addition to gap junctions [18].

Conclusions

The present study has shown altered gap junction distribution in coronary heart disease resulting from atrial dilation and atrial fibrillation. Remodelling of gap junctions might represent a modulating factor. Thus, it may be speculated that such factors might contribute to the persistence of atrial fibrillation.

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