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Zonula occludens–1 and connexin 43 expression in the failing human heart

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Abstract

Focal disorganization of gap junctional distribution and down-regulation of the major gap junctional protein connexin 43 are typical features of myocardial remodelling in the failing human heart. Increasing evidence indicates that connexin 43 interacts with zonula-occludens-1 (ZO-1), and it has recently been shown that ZO-1 promotes the formation and growth of gap junctional plaques. In the present study, distribution patterns of ZO-1 and connexin 43 were studied in normal and in heart failure patients using double-label immunohistochemistry and confocal microscopy. ZO-1 was found to be co-localized with connexin 43 at intercalated disks. Importantly, in patients with heart failure due to dilated or ischaemic cardimyopathy, areas of diminished connexin 43 expression were characterized by a markedly reduced ZO-1 staining. Based on these data it is concluded that in patients with heart failure, down-regulation of ZO-1 matches the diminished expression levels of connexin 43, suggesting that ZO-1 plays an important role in gap junction formation and gap junction plaque stability.

Keywords: cardiomyopathy • heart failure • intercalated disks • gap junctions • connexin 43 • zonula occludens-1

Focal disorganization of gap junctional distribution and down-regulation of connexin 43 are typical features of myocardial remodelling [1–3] that may play an important role in the development of an arrhythmogenic substrate in the failing human heart (reviewed in [4–6]). At present, the exact mechanism(s) of diminished connexin 43 levels and reduced gap junction size and number in different cardiac pathologies are still largely unknown. Among candidate mechanisms are those involving growth factors such as vascular endothelial growth factor [7], angiotensin II and endothelin 1 [8, 9], activation of the stress-activated protein kinase, c-Jun N-terminal kinase (JNK) [10]. Additional optional mechanisms are those involved in gap junction formation and stability, namely, the connexin interacting proteins such as tubulin [11], alphaII-spectrin [12], drebin [13] and zonula occludens-1 protein (ZO-1) [14].

In order to examine the distribution of ZO-1 in relation to that of connexin 43, tissue sections from three control hearts and explanted hearts from patients in end-stage heart failure due to dilated (five patients) or ischaemic cardiomyopathy (four patients) were analysed by immunohistochemistry and confocal microscopy. Dobule-labelling experiments were performed on frozen tissue sections using mouse anti-ZO-1 antibody (clone 1A12, Zymed, diluted 1:400) and a rabbit anti-connexin 43 polyclonal antibody (Zymed, diluted 1:100). Secondary antibodies used were Cy3-conjugated goat anti-mouse IgG (Chemicon, diluted 1:200) and fluorescein isothiocyanate (FITC)-conjugated

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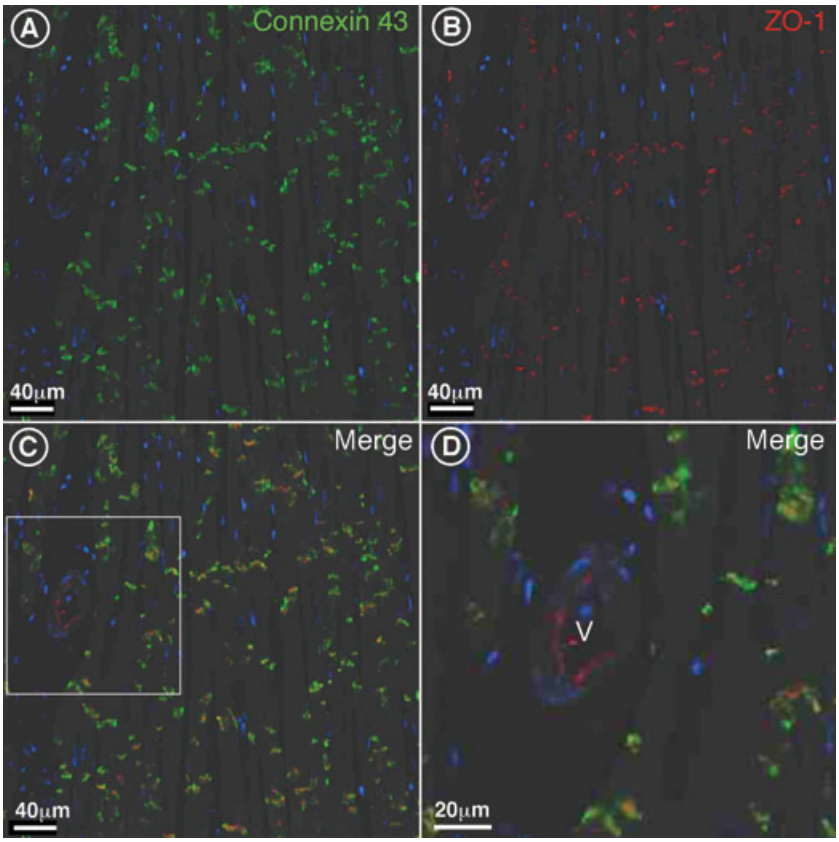

Fig. 1 Connexin 43 and ZO-1 distribution in normal human myocardium. Panel **A** and **B**: Connexin 43 and ZO-1, respectively. Panel **C**: A merge of Panels **A** and **B** illustrating that in myocytes ZO-1 is confined together with connexin 43 at the same intercalated disks. Note the homogeneous distribution of connexin 43 and ZO-1 throughout the myocardial tissue. Panel **D** is an enlargement of the boxed area in Panel **C** illustrating that ZO-1 is also confined to intercellular junctions of endothelial cells of intramural vessels (V). Nuclei are stained blue with DAPI.

goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, diluted 1:100), respectively.

Examination of tissue sections showed that in control human hearts, both ZO-1 and connexin 43, were homogeneously distributed throughout the myocardium and were confined to the same intercalated disks (Fig. 1A–C). ZO-1 immunostaining was also found in blood vessels representing tight junctions of endothelial cells (Fig. 1D). In human hearts in end-stage heart failure, there was a dramatic loss of ZO-1 staining. Figures 2A–C show that the loss of ZO-1 staining in the failing hearts coincided with a reduction of connexin 43 at the same intercalated disks, while the intensity of ZO-1 in blood vessels was found to be unaltered as compared with normal hearts (Fig. 2D).

These results are in conformity with recent studies using cultured ventricular myocytes, HeLa cells or lens epithelial cells in culture, which have elegantly demonstrated that ZO-1 regulates the cellular distribution of connexin 43 and consequently the size of gap junctions by controlling the rate of channel accretion at the gap junction plaque perimeter [14-16].

Conclusions

Based on these data it is concluded that in failing hearts, diminished ZO-1 expression coincides with reduced connexin 43 staining indicating that ZO-1 plays an important role in gap junctional formation and stability.

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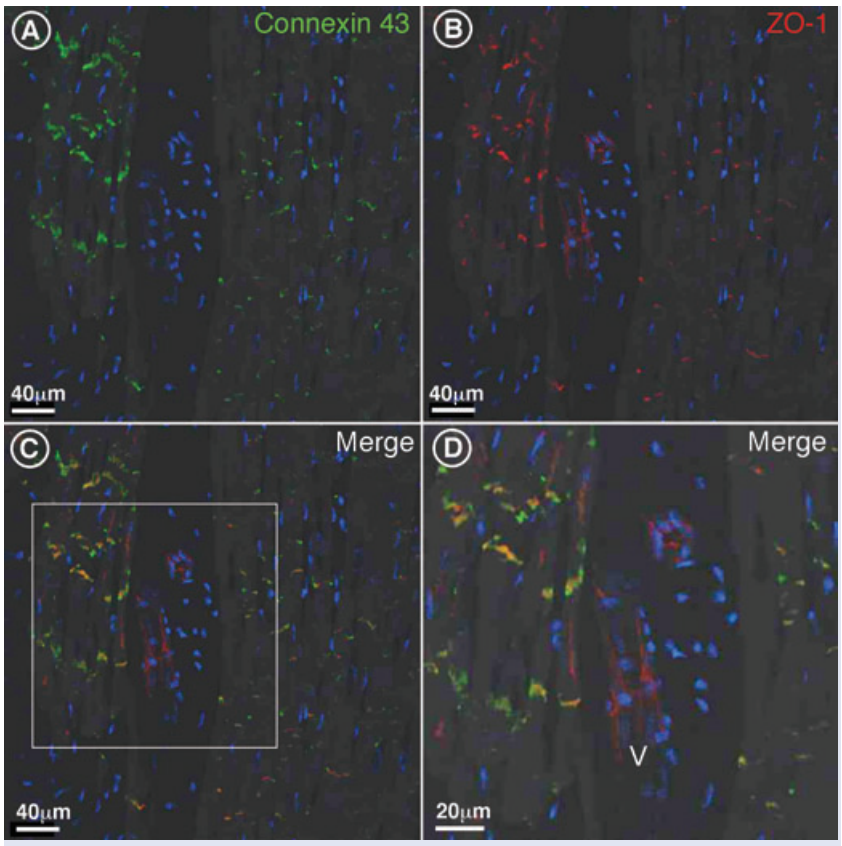

Fig. 2 Connexin 43 and ZO-1 distribution in a patient with dilated cardiomyopathy. Panel **A** and **B**: Connexin 43 and ZO-1, respectively. Note the patch of myocytes at the right side displaying reduced levels of connexin 43 and ZO-1. The patch is located next to myocytes at the left part of the micrographs which are intensely labeledlabelled for both connexin 43 and ZO-1. The difference between myocytes expressing high or reduced levels of connexin 43 and ZO-1 is even more pronounced in the superimposed image shown in Panel **C**. Panel **D** is an enlargement of the boxed area in Panel **C**, illustrating that ZO-1 is commonly confined to the intramural vessels (V). Note that, the intensity of ZO-1 in blood vessels in the diseased heart is apparently similar to the normal heart (compare Fig. 1D with Fig. 2D). Nuclei are stained blue with DAPI.

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