Atrial Structural Remodeling in Coronary Patients with and without Postoperative Atrial Fibrillation

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Structural atrial remodeling alters the substrate and spatiotemporal organization of the atrium in atrial fibrillation. In this paper we proposed: (a) qualitative and quantitative evaluation of structural changes in the atrial myocardium in coronary patients with and without postoperative atrial fibrillation; (b) assessment of the nature of structural changes: reversible and irreversible degeneration or dedifferentiation. The study included 20 coronary patients admitted at Cardiovascular Disease Institute in 2012. The patients were 14 men and 6 women aged between 36 and 74 years. The 20 patients, selected on clinical and laboratory criteria, were 10 patients with postoperative atrial fibrillation and 10 patients with postoperative sinus rhythm. Atrial tissue samples from patients in the atrial fibrillation group were compared with samples from patients who remained in postoperative sinus rhythm. Identifying lesions required optical microscopy examination, using special histological techniques and immunohistochemistry. Both groups developed degenerative and dedifferentiated lesions, but to different extents, values recorded in the postoperative atrial fibrillation being much higher than in the postoperative sinus rhythm patients.Our study indicated that hypertrophy, myocytolysis and fibrosis were defining injuries of the dedifferentiation process and accompanied degenerative alterations in both study groups. This implies that the morphological status of atrial tissue is a major determinant in the postoperative atrial fibrillation development and the improvement of preoperative metabolic status of myocardial cells may reduce the incidence of this common complication.

Key words: atrial remodeling, fibrillation, fibrosis, myolysis

Atrial structural remodeling (ASR) depends on cardiomyocyte and interstitial myocardial injuries. ASR includes myolysis and hypertrophy of cardiomyocytes, a reversible program of fetal protein gene re-expression, cell death through apoptosis and fibrotic-type changes of the extracellular matrix. These lesions cause a cascade of reactions that lead to atrial remodeling with structural, functional, electrical, and metabolic consequences [1-3].

Atrial remodeling was studied on animal models, in experimentally induced heart failure and atrial tachycardia [4].

Pathogenetically, atrial structural remodeling represents an adaptive response of cardiomyocytes, aimed to maintain homeostasis under the impact of external stress factors: tachycardia at a high depolarization rate together with volume and pressure overload. Specific stressors (ischemia, valvular disease, diastolic dysfunction, etc.) induce either functional adaptive reactions or maladaptive processes [5].

The remodeling type and its degree correlated with the duration of exposure to stress factors: (a) a 30 min exposure to stress produced changes at the ionic level that may be reversible; (b) a week exposure to stress caused usually reversible damages at cellular level (hibernation); (c) exposure to stress for weeks or months determined apoptosis and fibrosis at cellular and extracellular matrix level [6].

In ASR investigated during atrial fibrillation (AF), some authors reported reversible and irreversible atrial degenerative lesions, comprised of myocytolysis, apoptosis and fibrosis. Other authors described cardiomyocytes dedifferentiation processes [1] that did not associate with apoptosis. Such dedifferentiation lesions included myolysis, hypertrophy, and reorganization of protein expression to fetal-like patterns, such as á-smooth muscle actin (á-SMA) and desmin [7].

Until now, there are no complete studies of AF able to reveal all atrial lesion types or their various rates. Our investigation undertook an analysis of these reversible or irreversible possible disorders.

Experimental part

Materials and methods

The study included 20 patients hospitalized for coronary surgery in 2012, 14 men and 6 women aged between 36 and 74 years. All patients gave their consent to participate in the study prior to cardiopulmonary bypass surgery. We also had the Ethics Committee approval.

Patients were monitored for diagnosing postoperative atrial fibrillation. The 20 coronary patients (10 patients with postoperative atrial fibrillation - POAF and 10 with

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postoperative sinus rhythm - POSR) were selected on clinical criteria: absence of transitory POAF, no concomitant hyperthyroidism, and no valvular disease. Tissue samples from the right atrial appendages of the POAF group of patients were compared with samples from patients who remained in POSR.

The paper was accomplished by combining histopathological, immunohistochemical (IMH), morphometric and statistical studies.

Histological processing was performed in accordance with current standard protocols for tissue harvesting and fixation. Microscopic assessment used an optical microscope: Olympus CX 41. By routine (hematoxyline and eosine-HE), or special (collagen Sirius Red-SR) staining techniques, we identified cellular and extracellular damages: myolysis, hypertrophy and fibrosis. We also suspected degenerative lesions, including necrosis or apoptosis and dedifferentiation that required immunohistochemical confirmation.

Immunohistochemistry was applied according to standard protocols for IMH staining techniques performed on paraffin-embedded tissues. IMH study allowed us to diagnose accurately in cardiomyocytes (CMs) both dedifferentiated-type lesions and apoptotic-type degenerative lesions.

Quantification of lesions was performed by morphometry using a color image analysis system: QuickPHOTO MICRO 3.0. Data obtained were processed statistically and the results were expressed as mean values and percentages (small study group).

Myocytolysis means loss of myofilaments and appears as vacuolation of the cytoplasm. Morphometric quantification of myocytolysis related myocytolytic CM number to the total CMs number visualized on a high power field (HPF has a magnification of x400). We evaluated only myocytolytic CMs in which cytoplasmic vacuoles involved at least 25-30% of the cytosol. Myolysis was evaluated only in the cells containing the nucleus in the cross section plane.

Hypertrophy (HT) signifies an increase in CM size. Hypertrophy morphometric quantification was done by referring the hypertrophic CM number to the total number of CMs on the studied HPFs. Hypertrophy was determined by measuring CM transverse diameter only in cells displaying the nucleus in the cross section plane.

Fibrosis is the result of increased myocardial interstitium by fibrous tissue and was revealed by Sirius red staining. Morphometric quantification of fibrosis was performed by relating the fibrous interstitial area (stained in red with SR dye) to the studied histological section area.

Microscopically, we analyzed 10 histological fields on HPFs for each case. In myolysis and hypertrophy, the results were expressed as percentage or mean values for the number of myolytic or hypertrophic cells referred to the total nucleated cell number. The degree of fibrosis was evaluated by relating atrial fibrosis area to the entire studied area on HPF. Results were expressed as percentage of the fibrosis area related to the studied histological section area.

Dedifferentiation, consisting of re-expression of fetal proteins, was detected by IMH analysis of CM proteins, α-SMA and desmin. Quantification was achieved by relating the total number of CMs displaying positive reaction for a certain protein to the total number of the CMs in the studied histological sections. CMs were considered de-differentiated if they reexpressed markers specific to the fetal life and showed an attenuation of specific markers of adulthood.

Degenerative lesions were represented by apoptosis and contraction band necrosis. Apoptosis was suspected histologically and confirmed by IMH exam. CMs were characterized as apoptotic if the cell was shrunken having a pyknotic nucleus and condensed hypereosinophilic cytoplasm. Apoptosis was diagnosed by IMH with more accuracy. Quantification of apoptosis involved IMH detection of apoptosis associated proteins, Bcl-2 and P-53 respectively, and by the ratio between the positive-reaction CM number and the total CM number on HPFs in all histological sections considered.

Immunohistochemically we analyzed 10 histological sections at high magnification (x400 HPF) for each case. The results were expressed as percentage or mean values of the CM number with IMH positive reaction referred to the total number of nucleated cells in the area taken into account.

Results and discussions

Qualitative structural atrial changes observed in both study groups were cellular and extracellular lesions: CM myocytolysis, CM hypertrophy, nuclear alterations in myocytolytic CMs and interstitial fibrosis.

Structural changes were evaluated quantitatively by morphometry. In the quantitative study we quantified lesions on histological and IMH stained sections.

CM myocytolysis attained various degrees in the two studied groups. In POSR, myolysis interested about 1/5 cells (one fifth) out of the entire cell number (21.93%). In POAF, CM myolysis was slightly higher (28.61%). In POSR, we saw a uniform increase in CMs size without involvement of atrial architecture, while in POAF, various size CMs were present, with altered atrial architecture (fig. 1 a, b).

CM hypertrophy was observed in both POAF and POSR patient groups, although we noted different proportions between the two study groups (in POSR = 8.57%; in POAF = 9.07%)

Interstitial fibrosis was identified in both groups, having various degrees, but a higher proportion in POAF (23.41%) than POSR (16.76%). In POAF patients, we found wide collagenous septa separating isolated large groups of CM cells, which affected electrical conduction, while in patients with POSR a high degree of fibrosis was observed only in elderly patients (fig. 1 c, d).

IMH study allowed accurate diagnosis of de-differentiation and degenerative lesions suspected by us at histological examination made on usual or special stains. We studied immunohistochemically the dedifferentiated lesions by assessing cardiomyocyte proteins, α-SMA and desmin.

Normally, α-SMA is a contractile protein of fetal type, which is absent in adult type CMs. By dedifferentiation of CMs, a re-differentiation of this fetal-type protein (α-SMA) in adult-type protein (desmin) occurs. In adult atrial CMs, we found a positive reaction for α-SMA at the periphery of myolytic CMs. The degree of dedifferentiation was slightly higher in the POSR group (16.03%) than the POAF group (14.36%), suggesting more significant adaptive changes in patients with sinus rhythm (POSR) (data not shown).

Desmin is a protein characteristic of adult type phenotype of the cardiomyocytes. In the process of CM dedifferentiation we observed the reduction of desmin positive reaction expressed in adult type CMs in both groups. This aspect was revealed at the periphery of myolytic CMs and at the level of the intercalated disks. Positive reaction to desmin was somewhat lower in POAF (26.07%) than in POSR (29.41%), denoting greater loss of the CM contractile function in AF (data not shown).

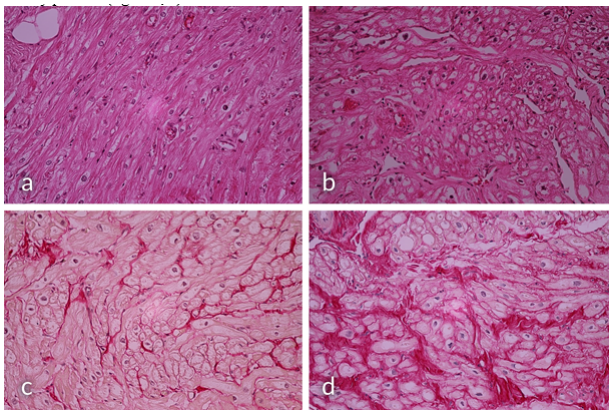

Fig. 1. (a) POSR, (b) POAF - myocytolysis (HE, x40); (c) POSR, (d) POAF - interstitial fibrosis (SR, x40)

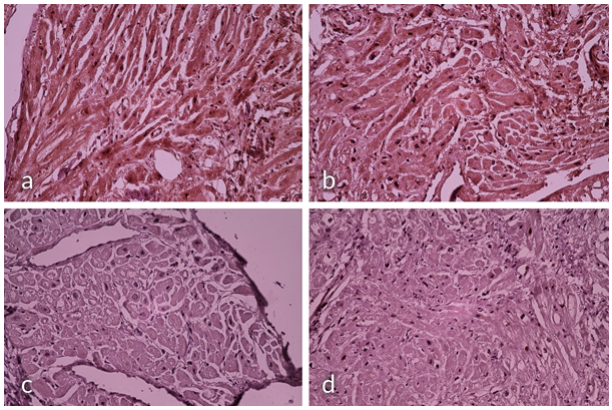

Fig. 2. (a) POSR, (b) POAF - atrial cardiomyocytes (Bcl-2, x40); (c) POSR; (d) POAF - atrial cardiomyocytes (P53, x40)

Firstly, we detected distinct degenerative changes by evidentiating apoptosis on histological samples, and then we have confirmed them immunohistochemically. Identification was made by evaluation of apoptosis - associated proteins, Bcl-2 and P-53, respectively. Apoptosis was absent in normal myocardium (Bcl-2 was absent). We found cytoplasmic and nuclear positive reaction in few cardiomyocytes for Bcl-2, indicating apoptotic tendencies of CMs. We found minor differences between the two groups (POSR - 1.19%; POAF - 1.49%) (fig. 2 a, b) as to the proportion of CMs with positive reaction.

Normal myocardium lacks P-53. By studying P-53 protein, we pointed out an increased nuclear expression of p-53 showing that cardiac myocytes undergo apoptosis, a phenomenon observable in both normal and dedifferentiated CMs. We did not notice major differences in the proportion of CMs with positive reaction between the two groups (POSR - 1.01%; POAF - 1.39%) (fig. 2 c, d).

The study indicated that patients with coronary artery disease developed deep structural changes in atrial myocytes.

Morphometric data showed remarkable differences between patients with POSR and POAF such as the appearance of large vacuoles in CMs, suggesting the role of associated factors, including the patient's metabolic status, in AF development [8]. In our series, patients' atrial myocytes predominantly displayed myolytic changes. AF development was accompanied by cell size enlargement, an aspect evident in both groups of patients, yet with a higher extension in POAF due to involvement of more CMs.

Vacuolation was shown to occur during reversible myocardial damage and was suggested to be a predictor of vulnerability. In the literature there are two opposing views on the nature of the lesions in ASR of either degenerative or dedifferentiated types. It should be outlined that a clear distinction exists between dedifferentiated and apoptotic cardiomyocytes [9, 10].

Some authors claim that cell degenerative lesions including apoptosis are not generally observed in dedifferentiated CMs. Apoptosis is an irreversible damage leading to cell loss which is replaced by interstitial fibroblast proliferation, and fibrosis [5, 11]. We believe that cardiomyocyte apoptosis may occur in dedifferentiated cells when they are unable to adapt to a further decrease in oxygen supply.

Impaired cardiac atrial function in patients with coronary heart disease leads to changes in structural CM proteins. Such changes are described as adaptive reactions of dedifferentiation, indicative of fetal phenotype. Intra- and extra- cellular changes contribute to changes in the electrical circuitry rendering the atrium more vulnerable to the development of AF [6, 12].

Conclusions

Our study detected a wide range of atrial structural changes, including dedifferentiation and degenerative lesions. Dedifferentiation and degenerative lesions coexisted. In fibrillating atria, the myolytic myocytes are in a dedifferentiation state similar to that of immature CMs. Dedifferentiation may be the best way for CMs to survive in case of prolonged exposure to adverse conditions.

Limits of the study

It is clear that AF associated with a significant increase in cell size and loss of the contractile apparatus in myolytic CMs. But under certain circumstances, myolysis is both a degenerative and an adaptive lesion. It is not clear whether alterations observed in AF can be classified as reversible or irreversible. To solve this problem, experimental studies are needed focused on the progression of changes and their potential reversibility.

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Manuscript received: 15.01.2016