

Morphometry of Human Myocardium in Senile Individuals

José Antonio Cardoso, Ana Elisa Toscano, Tetsuo Tashiro, Cláudio Antonio Ferraz de Carvalho, Sílvia Regina Arruda de Moraes

Universidade Federal de Pernambuco e Instituto de Ciências Biomédicas - USP - Recife, PE – São Paulo, SP - Brazil

OBJECTIVE

To carry out a quantitative assessment in human myocardium cells of senile individuals, in right, left and septal ventricular regions.

METHODS

Five hearts from corpses of individuals without heart diseases, of both sexes, with age between 67 and 87 years old were used. The following parameters were assessed: myocyte unit cross section area (myoc. a_o); myocyte unit perimeter length (myoc. l_o); myocyte unit volume (myoc. v_o); myocyte volumetric density (myoc. V_v); number of myocytes per volume unit (Nmm⁻³myoc.). The t-test of Student was used in statistic analysis.

RESULTS

The analysis of differences ($p < 0.05$) among right (RV), left (LV) and septal (S) ventricular regions of human heart showed that myoc. a_o values were lower in RV ($1.51 \pm 0.10 \mu\text{m}^2$) and in S ($1.55 \pm 0.07 \mu\text{m}^2$) in relation to LV ($1.84 \pm 0.24 \mu\text{m}^2$). Values of myoc. l_o were also shown lower in S ($5.11 \pm 0.46 \mu\text{m}$) comparing to LV ($6.2 \pm 0.97 \mu\text{m}$). Likewise, myoc. v_o and myoc. V_v showed lower values in RV ($88.75 \pm 25.37 \mu\text{m}^3$; $0.39 \pm 0.03\%$) in relation to LV ($122.41 \pm 16.31 \mu\text{m}^3$; $0.41 \pm 0.01\%$).

CONCLUSION

Results obtained show that there may be changes in dimensions of left ventricular wall myocyte cell during senescent stage. However, those differences are subtle and seem to mean the adjustment of tissue to functional changes that install along life.

KEY WORDS

Myocytes, cardiac, aging, myocardium/cytology.

The understanding on heart aging process is important for a better knowledge of pathological processes that more commonly settle in senile individuals. However, one of the greatest difficulties found in the study of aging effects on cardiovascular system is of isolating and identifying the effects of normal or physiological aging process on those related with the presence of associated specific pathological statuses¹. Besides, other difficulties are reported when one wishes to carry out an investigation of the aging physiological process alone, such as the lifestyle of each individual (level of physical activity and stress, alcohol ingestion and smoking). All those factors may contribute to unleash changes in cardiovascular function in the course of individual's aging, although its specific contribution is very difficult to quantify^{2,3}.

The study of aspects pertaining to morphometry and stereology of human myocardium is important for the understanding of compensatory mechanisms observed at that stage of life. However, there are few data in literature on morphometric aspects of the heart during senescent stage, although this muscle is widely studied, under qualitative point of view in mammals, especially in men, in several pathological processes⁴⁻¹².

The present work aims at comparing quantitative differences in human myocardial cells from right and left ventricular and septal wall regions in senile individuals, in order to obtain information that can provide a better comprehension on pathological processes that are very frequent in the senescent stage of the individual.

METHODS

Hearts from five corpses of individuals of both sexes, with ages ranging from 67 to 87 years old, coming from the Departamento de Anatomia (Anatomy Department) of Universidade de São Paulo were studied.

The hearts were catheterized through left coronary artery, washed with salt solution at 9% and perfused with fixative solution of paraformaldehyde at 10% in 0.1 M phosphate buffer (pH 7.3), until fluid outflow through coronary sinus was observed. Subsequently, the hearts were immersed in the same fixer during 48 hours. Smaller fragments of myocardium from right (RV) and left (LV) ventricular walls, on the level of sternocostal side, and from interventricular septum (S) muscle wall were collected and placed in the 9% paraformaldehyde fixative solution for five days. Then, they were submitted to a slow dehydration process, in a methyl benzoate solution and through the mixture of benzol-paraffin in growing proportions after dehydration and finally, included in paraffin. After inclusion, 6 μm -thick histological cuts were obtained in a JB4A-SORVALL microtome, with heart muscle fibers sectioned approximately parallel to their larger axis. Cuts perpendicular to their larger axis were carried out and dyeing through ferric hematoxylin

method. Thirty microscopic fields, randomly chosen, were analyzed in each cut.

For histocytometric analysis^{13,14}, an ocular with 100-point test-system¹⁵ in optical microscope, with a final enlargement of 1,000x. The following parameters were analyzed: myocyte unit cross section area (myoc. a_o); myocyte unit perimeter length (myoc. l_o); myocyte unit volume (myoc. v_o); myocyte volumetric density (myoc. V_v); number of myocytes per volume unit ($N_{mm} \text{ } ^3\text{myoc.}$).

Statistical analysis was carried out, comparatively, among the three myocardial regions (LV, RV and septal wall). For the comparison of pairs of means, the t-test of Student was used. All tests were carried out with a significance level of 5%.

RESULTS

Histocytometric analysis of myocyte cells in three human myocardium regions of senile individuals demonstrated that the myocyte unit cross section area was smaller ($p < 0.05$) in RV ($1.51 \pm 0.10 \mu\text{m}^2$) and in S ($1.55 \pm 0.07 \mu\text{m}^2$) when compared with LV ($1.84 \pm 0.24 \mu\text{m}^2$). Myocyte unit perimeter length, in the same group, was also smaller ($p < 0.05$) in S ($5.11 \pm 0.46 \mu\text{m}$) in relation to LV ($6.2 \pm 0.97 \mu\text{m}$). Likewise, myocyte unit volume and myocyte volumetric density showed lower values ($p < 0.05$) in RV (88.75 ± 25.37 ; $0.39 \pm 0.03 \mu\text{m}^3$) in relation to LV ($122.41 \pm 16.31 \mu\text{m}^3$; $0.41 \pm 0.01\%$) (tab. 1).

DISCUSSION

Structural changes observed in human myocardium, due to aging process, are not completely elucidated. It is known that at senescence stage a reduction in the number of cardiomyocytes takes place, followed by their hypertrophy^{16,17}, causing a decrease of heart functional reserve, which may favor ventricular dysfunction and heart failure at that stage in life^{17,18}. Understanding of histocytometric changes of cardiomyocytes in different areas of aging heart is important for the comprehension of changes taking place in the heart at that stage in life. Concerning the myocyte cross section area, an experimental study with normotensive rats¹⁹ demonstrated the existence of a significant decrease of myocyte section area in animals with age between 18 and 24 months. However, those authors did not specify the heart areas used as sample for that study. Other authors demonstrated, in human hearts, that the myocyte cell area close to endocardial region was shown larger than in the area near epicardial regions^{5,20}. In the present study, a research related to the myocyte cell area with relation to its position in different heart coats was not carried out, but taking as reference the heart regions, in which a decrease of myocyte cross section area was only observed in right ventricular and septal regions.

Table 1 – Morphometric analysis of myocytes in senile individuals

		Parameters				
		Myoc. a_o (μm^2)	Myoc. l_o (μm)	Myoc. v_o (μm^3)	Myoc. V_v (%)	$Nmm^{-3}myoc.$ ($myoc/mm^3$)
Senile (n-5)	RV	1.51 \pm 0.10*	5.16 \pm 0.17	88.75 \pm 25.37*	0.39 \pm 0.03*	2,493,591.4 \pm 731,577.34
	LV	1.84 \pm 0.24	6.2 \pm 0.97	122.41 \pm 16.31	0.41 \pm 0.01	2,660,843.3 \pm 319,464.03
	S	1.55 \pm 0.07*	5.11 \pm 0.46*	94.04 \pm 13.02	0.40 \pm 0.01	2,702,357.7 \pm 34,468.25

*Myoc. a_o – myocyte unit cross section area; Myoc. l_o – myocyte unit perimeter length; Myoc. v_o – myocyte unit volume; Myoc. V_v – myocyte volumetric density; $Nmm^{-3}myoc.$ – number of myocytes per volume unit. RV – right ventricular region; LV – left ventricular region; S – septal region. Values are shown as mean \pm SD. * $p < 0.05$ (t-test of Student).*

Senile individual myocytes showed a decrease in length of unit perimeter in septal region in relation to left ventricular region. The myocyte length is very difficult to obtain dimension in myocyte cell, especially in adult myocardium, due to the difficulty of obtention of longitudinal cuts in the cell²¹, a fact that can justify the scarcity of literature data on the length of myocyte unit perimeter, either in experimental works or works with human beings.

When the values of the three heart regions were analyzed, a decrease in myocyte unit volume in right ventricle in relation to the left ventricle, and a decrease of myocyte volumetric density in right ventricle, when compared to the left ventricular region, was found. However, data on those histocytometric parameters in different ventricular regions and on septal wall at senile stage were not found in the literature.

There was no difference between the groups in the analysis on the number of myocytes per volume unit. However, authors observed that the decrease in cardiac mass, due to a continuous loss of myocytes in ventricular regions, would take place as an age functional aspect, and it would give place to external changes in wall thickness of both ventricles^{1,22,23}. Despite not having quantified, in this study, the number of myocyte cells in each region, values

from most histocytometric parameters observed in the left ventricle, compared to the other two studied regions, may suggest a compensatory mechanism of cell loss.

Changes in ventricular structure seem to cause myocyte cell hypertrophy, as observed in hypertension or vascular disease, whereas hyperplasia or “pseudo-hypertrophy” results from reoccupation of myocyte cell areas by non-contractile tissue, as observed in ischemic heart disease^{24,25}. Myocardial hypertrophy, for its turn, is the attempt of adjustment of the heart to work overload, evolving to heart failure when the adjustment process is depleted²⁶.

The normal aging process would be, then, associated to a series of changes. However, those changes would be gradual and relatively moderate, with the possibility to decrease aging heart capability in adjusting to the stress imposed from settlement of some cardiovascular disease²⁷. Changes in dimensions of left ventricular wall myocyte cell take place during the senescent stage of the individual. That may be related to the reduction of the number of those cells and the probable hypertrophy of remaining cells. However, those differences are subtle and seem to mean the adjustment of the tissue to functional changes settling along the life.

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