

# Distribution of cardiomyocytes in cell cycle during aging

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## ABSTRACT

Cell cycle activity is an intrinsic component of cardiac differentiation and morphogenesis. In this study isolated mice hearts were used to investigate the effects of age on cardiomyocytes' cell cycle under normal physiological conditions *in vivo*. Number of diploid, tetraploid, polyploid (octaploid, 16n and 32n), apoptotic nuclei and nuclei in S phase were counted by a Becton Dickinson (Mountain View, CA) FACScan flow cytometer. It has been considered, that in all studied age groups during of cell cycle are identical. However, in adults comparatively to juvenile mice simultaneously increases the amount of polyploid and apoptotic nuclei. At the same time the quantity of apoptotic nuclei remains high in senescent mice. Herewith, the amount of polyploid nuclei decreases in this group. Also, according to our experimental data the capacity of re-initiation of DNA synthesis in the heart is low. However, the fact of DNA synthesis *per se* seems to be insufficient to drive adult cardiomyocytes through cell division. So, we can consider, that the heart has low regenerative potential.

**KEYWORDS:** *cardiomyocyte, cell cycle, apoptosis*

**C**ardiovascular disease is a leading cause of death worldwide. It can be initiated by multiple factors; in recent years it has emerged that a major contributory factor to its initiation and progression is the loss of cardiomyocytes. Adult cardiomyocytes are terminally differentiated cells and, once destroyed, are rarely replaced. Thus, their loss can contribute to the functional decline of the myocardium leading to heart disease. [5].

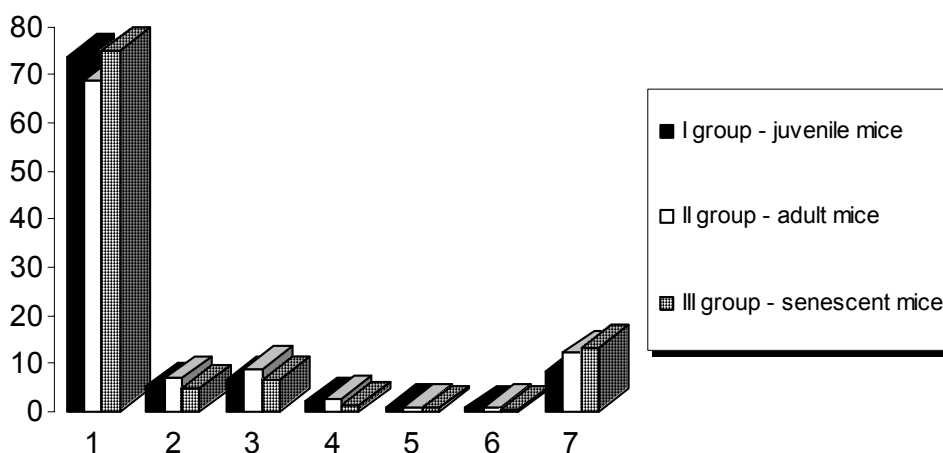
It is known that the myocytes of the adult mammalian heart are considered unable to divide. Instead, mitogens induce cardiomyocyte hypertrophy. [4]. Cardiac myocytes are adapted to increased work and compensate for disease exclusively through hypertrophy. [7].

Loss of cardiocytes during myocardial hypertrophy can lead to progressive dysfunction in hearts with chronic hemodynamic overload. Such myocardial hypertrophy is morphologically associated with progressive myocardial degeneration, as reflected by cardiocytic ultrastructural changes and numerous atrophic cardiocytes found among the hypertrophic cells. Loss of these cardiocytes probably represents a process of sequential cardiocyte elimination

in association with cell degeneration or atrophy, rather than an independent concomitant process. However, little is known of the possible mechanisms for cell elimination in the course of human myocardial remodeling that is generally thought to occur in hypertrophied hearts. Since there is no inflammatory response or other indication of cardiocytic necrosis in the hypertrophied myocardium of chronic hemodynamic overload, apoptosis is a plausible alternative explanation for the cardiocyte loss. [10].

Loss of myocytes in the aging heart may be due to necrosis and apoptosis. Apoptosis is a highly regulated form of cell death that is characterized by specific morphological, biochemical, and molecular events. It is essential to normal development of multicellular organisms and is involved in cell turnover and remodeling in healthy tissue. Apoptosis also plays a critical role in removing unwanted and potentially dangerous cells such as tumor cells and cells infected by viruses. [9].

The aim of present work was to investigate the age related changes of the cardiomyocytes' cell cycle in white mice.



1 - diploid (G0/G1); 2 - S phase; 3 – tetraploid; 4 – octaploid; 5 – polyploid - 16n; 6 - polyploid - 32n; 7 – apoptotic

**Fig.1** Distribution of cardiomyocytes' nuclei in cell cycle during aging.

## MATERIALS AND METHODS

**Animals:** 18 white mice were used in this study. According to the age the animals were distributed in three groups: I group – juveniles (6 mice, 2-months old,  $18,0 \pm 2,0$  g body weight), II group – adults (6 mice, 10-months old,  $25,0 \pm 2,0$  g body weight), III group – senescents (6 mice, 18-months old,  $30,0 \pm 2,0$  g body weight). The animals were maintained at 18-22°C temperature and light-controlled environment with a 12:12-h light-dark cycle and provided with food and water ad libitum.

**Methods:** The animals were anesthetized with ether narcosis and were killed by the method of decapitation. The chest was opened and heart was removed. Heart tissue was homogenized with a glass-Teflon Potter homogenizer in the 2,2 M succrose solution prepared on the phosphate buffer (pH 7,4). Nuclei were collected by centrifugation of the homogenate at 18000 x g for 45 min. Received pure of nuclei was suspended in 3 ml TMS solution and centrifuged at 3000 x g during 10 min. 70% ethanol was added to the pellet and samples were incubated during 24-h at 4°C. Then 20 ml of RNase (10 mg/ml) was added and samples were incubated during 30 min at 37°C. After 1 ml of EB staining solution was added, samples were incubated during 30 min at 37°C.

Number of diploid, tetraploid, polyploid (octaploid, 16n and 32n), apoptotic nuclei and nuclei in S phase were counted by a Becton Dickinson (Mountain View, CA) FACScan flow cytometer.

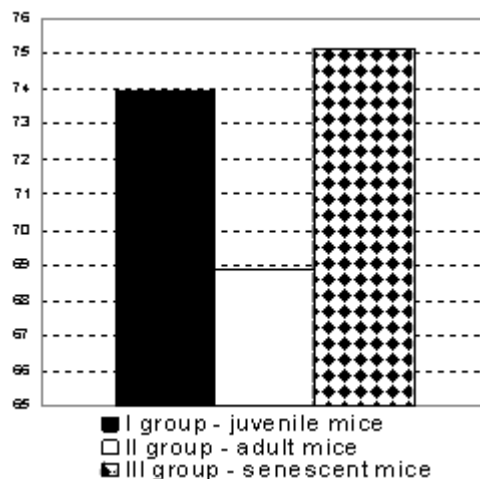
Statistical analyses were performed using Student *t* test for independent samples. Probability (*p*) values of <0,05 were considered to be significant. All data are expressed as mean (standard deviation).

## RESULTS AND DISCUSSION

Existing data, about distribution of different ploidy cardiomyocytes at different stages of age are controversial. Vasil'eva et al. [1988] have shown, that in human cardiac myocytes the percentage of DNA classes for diploids, tetraploids (both 4c and 2c X 2), octaploids (8c, 4c X 2 and 2c X 4), hexadecaploids (16c, 8c X 2 and 4c X 4) and 32c cells is 2%, 32%, 53%, 12% and less than 0.5%, respectively. In adults binuclear cells comprise about 65% of the myocytes; the main class is 4c X 2 [11]. On the other hand Aref 'eva et al. [1985] have indicated, that more than 90% of rat myocytes are normally mononuclear diploid cells [1].

According to our experimental data, in all studied age groups the heart demonstrate a high proportion of cells in G0/G1 (diploid) phase (68,89%-75,16%) ( $p < 0,0001$ ). Apoptotic nuclei proportionally are most numerous after diploid nuclei (8,27%-13,12%) ( $p < 0,05$ ). Than one after another decreases the quantity of tetraploid (6,53%-8,77%), nuclei in S phase (4,88%-6,85%), octaploid (1,48%-2,78%), polyploid-16n nuclei (0,73%-1,0%) ( $p < 0,05$ ). Only the amount of 16n and 32n (0,65%-1,1%) (polyploid) nuclei are identical ( $p > 0,05$ ) (Fig.1.).

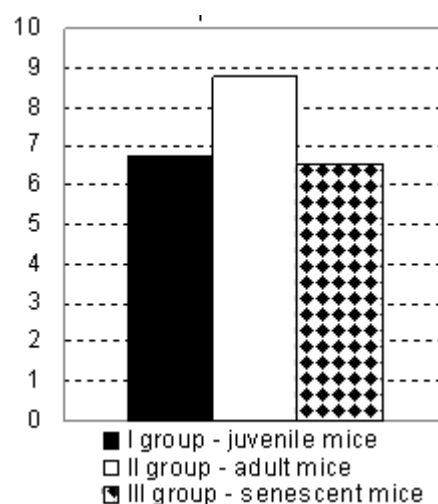
Our experimental study have shown, that under normal conditions cardiomyocytes in mice contained mostly diploid nuclei. However, the amount of diploid nuclei varies from one age group to another. This parameter is significantly high in juvenile mice. It decreases in adults ( $p < 0,05$ ) and again increases in senescent mice ( $p < 0,05$ ) (Fig.2).



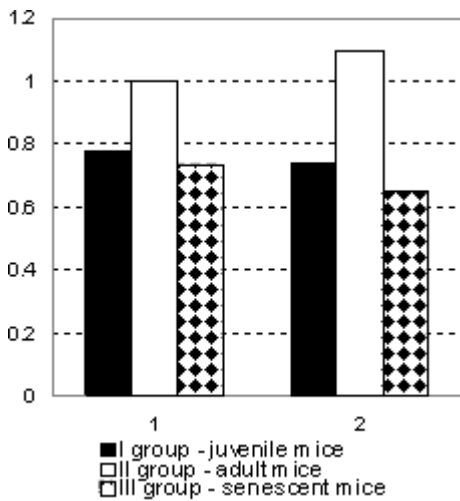
**Fig.2** Age related changes in amount of mice cardiomyocytes' diploid nuclei.

In newborn mice, up to 90% of the myocytes are diploid cells, then polyploidization occurs [3]. The stable ratio of ploidy classes was observed from 3 weeks to 1 year of age in mice by Brodsky et al. [1985]. The main class was always binucleate 2c X 2, comprising approximately 80% of the entire population. Nor were many mononucleate tetraploids (4c) and octaploids of different types (8c, 4c X 2, 2c X 4, 2c + 2c + 4c) observed. An insignificant number of hexadecaploids (16c and 8c X 2) could be found in some animals.

Obtained data have indicated, that in adults comparatively to juvenile mice the amount of diploid nuclei decreases ( $p < 0,05$ ), whereas, the amount of polyploid (tetraploid, 16n and 32n) nuclei increases ( $p < 0,05$ ). At the same time the quantity of polyploid (tetraploid, 16n and 32n) nuclei decreases in senescents ( $p < 0,05$ ) and are identical at the same parameters in juvenile mice ( $p > 0,05$ ) (Fig.3,4).

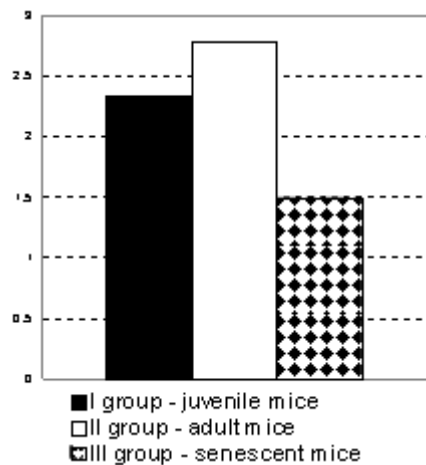


**Fig.3** Age related changes in amount of mice cardiomyocytes' tetraploid nuclei.

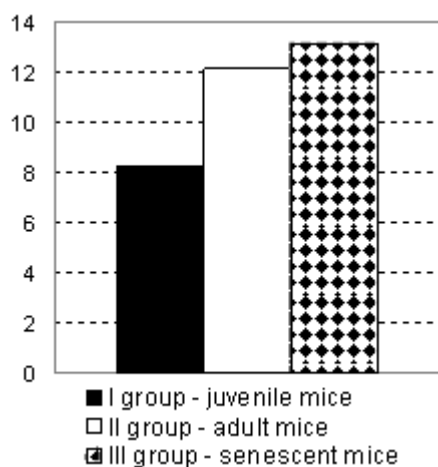


1. Polyloid 16n  
2. Polyloid 32n

**Fig.4** Age related changes in amount of mice cardiomyocytes' polyloid 16n and 32n nuclei.



**Fig.5** Age related changes in amount of mice cardiomyocytes' octaploid nuclei.



**Fig.6** Age related changes in amount of mice cardiomyocytes' apoptotic nuclei.

Some authors have hypotheses, that in mouse heart the formation of 4c mononuclear cells is accomplished not by acytokinetic but via other types of mitotic arrest; this may be due, for example, to a block in the pro- or metaphase. Only very rare cases of cytotomy were detected and the number of newly formed 2c cells was very low. It is concluded that cell multiplication is practically arrested at this period of life, and growth of the ventricular mass is due to polyploidization of virtually all cycling cells, while their number remains unchanged [2].

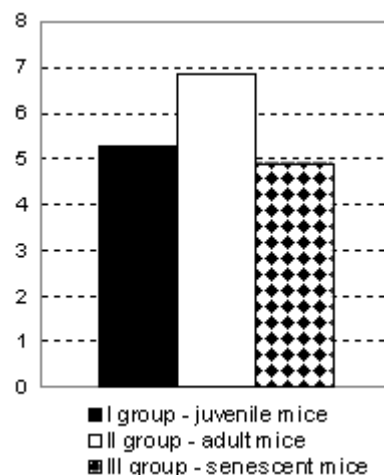
According to our experimental data, there is no significant difference between the amount of octaploid nuclei in juvenile and adult mice ( $p > 0,05$ ). This parameter decreases in senescent mice ( $p < 0,05$ ) (Fig.5).

Our experiments have shown that in all age groups heart contains the different amount of apoptotic nuclei. This parameter was significantly low in juvenile mice, but it increases soon in adults ( $p < 0,05$ ) and remains high in senescent mice ( $p > 0,05$ ) (Fig.6).

It is well known that DNA-ploidy is useful independent prognosticator of malignancy [12]. Cell death by apoptosis is a highly conserved evolutionary process for deleting senescent, damaged, redundant and deleterious cells from the organism. Apoptosis also plays a critical role in removing unwanted and potentially dangerous cells such as tumor cells and cells infected by viruses [9].

It is remarkable, that in adults simultaneously increases the amount of polyloid and apoptotic nuclei. At the same time the quantity of apoptotic nuclei remains high in senescent mice. Herewith, the amount of polyloid nuclei decreases in this group.

Nakagawa et al. [1988] have shown, that in mice the cardiomyocytes synthesised DNA most actively in the first 14 days after birth; in various parts of the heart it seen in about 8.8%. Twenty days after birth DNA synthesis of the cardiomyocytes rapidly decreased, it being evident in less than 0.2%. From 100 to 200 days DNA synthesising cardiomyocytes were seen occasionally in the subendocardial region of the left ventricle (index 0.04%) [8]. At the same time Kishore et al. [2002] generally have



**Fig.7** Age related changes in amount of mice cardiomyocytes' nuclei in S phase.

accepted that, adult cardiomyocytes retain some capacity to synthesize DNA. However, there is considerable debate regarding the frequency at which this occurs and if re-initiation of DNA synthesis necessarily leads to cell division. When considering the intrinsic proliferative capacity of adult cardiomyocytes, it is important to reiterate that DNA synthesis does not necessarily result in genome duplication, that genome duplication does not necessarily result in karyokinesis, and that karyokinesis does not necessarily result in cytokinesis [6].

Obtained data have indicated, that in the heart in all studied age groups there are distinct amount of nuclei in S phase and there is no significant difference between this parameters in juvenile, adult and senescent mice ( $p > 0,05$ ) (Fig.7).

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Thus, according to our experimental data, the capacity of re-initiation of DNA synthesis in the heart is low. However, the fact of DNA synthesis per se seems to be insufficient to drive adult cardiomyocytes through cell division. So, we can consider that the heart has low regenerative potential.

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## Возрастные особенности распределения кардиомиоцитов в клеточном цикле

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### РЕЗЮМЕ

Изучены возрастные особенности распределения кардиомиоцитов в различных фазах клеточного цикла. Определялись ядра, находящиеся в синтетической (S) фазе клеточного цикла, а также количество диплоидных (2n), тетраплоидных (4n), полиплоидных (8n, 16n, 32n), апоптотических ядер с помощью динамического цитометра. Особенности течения клеточного цикла изучались у ювенильных, зрелых и старых животных. Установлена идентичность течения клеточного цикла у животных в пределах изученных возрастных групп. Однако отмечается тенденция к увеличению количества апоптотических клеток у животных старческого периода, а количество ядер, находящихся в синтетической (S) фазе клеточного цикла, остаётся неизменным.

**Ключевые слова:** кардиомиоциты, клеточный цикл, апоптоз