EFFECT OF INJURY ON THE COURSE OF MITOSIS IN CARDIOMYOCYTES OF YOUNG RATS

G. B. Bol'shakova

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During the first months of life mitotic activity of rat cardiomyocytes gradually declines [1, 3, 5]. This decline is accompanied by lengthening of the cell cycle and disturbances of mitosis: defects of spindle formation and function, cessation of cytotomy [5, 8, 9]. After injury to the myocardium of the left ventricle in early postnatal ontogeny mitotic activity of the myocytes of the atria, auricles, left subepicardial zone, and trabecular myocardium of both ventricles rises sharply [2, 7].

The object of the present investigation was to determine what causes the post-traumatic rise in the mitotic index – an increase in the number of proliferating cardiomyocytes or changes in the course of mitosis.

Ethanol was injected into the wall of the left ventricle of noninbred albino rats aged 1, 7, 14, and 30 days. The hearts were investigated on the 2nd, 4th, 7th, 14th, and 30th days after the operation. Intact animals of the same age served as the control. Each group consisted of 8-10 rats. The hearts were subjected to the usual histological treatment and sections 5-7 μm thick were cut longitudinally through the whole heart. The mitotic index was determined in 3000-4000 muscle cells and the phases of mitosis were analyzed in the following regions of the heart: left and right atria and their auricles, subepicardial zones, compact and trabecular myocardium of both ventricles.

The relative percentage of prophase: metaphase: anaphase: telophase (P:M:A:T) were determined in the whole heart and in its separate zones.

The numerical results were subjected to statistical analysis using the chi-square criterion. Differences were considered significant at the $P < 0.05$ level.

EXPERIMENTAL RESULTS

During normal postnatal development of the heart muscle the relative duration of the phases of mitosis changed (Fig. 1). Whereas in animals aged 3 and 5 days there was no significant difference between the P:M:A:T ratio, in rats aged 5 and 8 days, and also in rats aged 8 and 9 days these values differed at the $P < 0.01$ level. With age a tendency was observed for the fraction of metaphases to increase, only to fall again in animals aged 11 and 15 days. The reason for this may be that at this age mitosis in the compact myocardium, where the increase in the fraction of metaphases was previously more marked [7], has almost ceased. On the 16th day of life the fraction of metaphases increased again. Anaphases and telophases also were absent, indicating possible disturbances in the course of cell division, such as death of some of the cells during mitosis or blocking of karyokinesis, leading to the formation of polyploid nuclei. A fact which deserves special attention is that the changes described above, accompanying the decline in proliferation of cardiomyocytes during growth of the heart muscle, did not take place simultaneously in different parts of the heart.

Mitotic activity declined most rapidly in the more highly differentiated compact myocardium of both ventricles. Absence of anaphase and telophase was observed in this part on the 9th day of life (in the left ventricle the ratio P:M:A:T was 9.4:90.6:0:0 and in the right 15.4:84.6:0:0 respectively), whereas in the right subepicardial zone a similar state of affairs was not observed until the 11th day of life (one prophase and nine metaphases in all hearts in the group), and in the right auricle not until the 15th day (10 prophases and 11 metaphases). This confirms the view [5] that mitotic activity of the cardiomyocytes is inhibited when the "critical weight" of contractile proteins has accumulated.

Laboratory of Growth and Development, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.)
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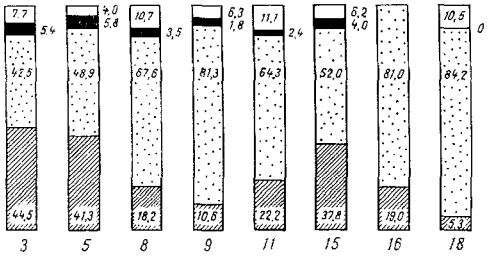

Fig. 1

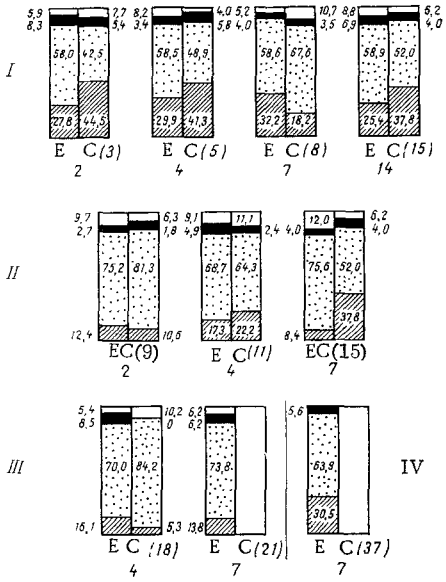

Fig. 2

Fig. 1. Overall ratio P:M:A:T (numbers in columns from below upward in %) in normal postnatal ontogeny. Numbers below columns show age of animals (in days).

Fig. 2. Overall ratio P:M:A:T (numbers in columns from below upward, in %) in different age groups after injury to myocardium. E) Experiment, C) control; I) injury inflicted at age of 1 day, II) 7 days, III) 14 days, IV) 30 days. Numbers below columns show number of days after injury; numbers in parentheses show age of control animals.

As was shown previously, proliferation in the injured growing heart declines more slowly than in the intact heart [2, 7]. In this case the ratio between the phases of mitosis arising in the myocytes after injury to the left ventricle was found to be more constant (Fig. 2).

Anaphases and telophases were found in the hearts of animals of all experimental groups, indicating completion of karyokinesis.

The ratio between the phases of mitosis in the experimental groups, unlike in the control groups, was constant. In animals injured at the age of 1 day the P:M:A:T ratio did not differ significantly in any of the groups. In rats injured at the age of 7 and 14 days the percentage of metaphases was greater, but no significant differences likewise were found between the relative percentages of the phases of mitosis at all times after injury.

Constancy of the P:M:A:T ratio was clearly observed in groups of animals injured at the stage of postnatal ontogeny when proliferation of the cardiomyocytes in the control had ceased. In 14-day animals on the 4th day after injury the P:M:A:T ratio differed from the control at the $P < 0.01$ level, on the 7th day proliferation in the control was almost completely absent and the P:M:A:T ratio in the experimental groups did not differ significantly from that in 9-day rats of the control group. In animals injured at the age of 30 days, mitotic activity of the cardiomyocytes on the 7th day after injury in the control was already almost completely blocked, but in the experimental group the ratio P:M:A:T, which was 30.5:63.9:5.6:0, indicated the normal course of mitosis.

The ratio between the phases of mitosis in different parts of the heart did not differ appreciably, evidence of stability of the character of the course of mitosis in the nuclei of the cardiomyocytes.

After injury to the heart in young rats a true increase in proliferation of the cardiomyocytes thus takes place. Mitoses appearing in the injured heart resemble more closely as regards the ratio between their phases mitoses in the hearts of animals in the initial stages of postnatal ontogeny. A resemblance of this sort may probably also be observed in the heart injured at a later age. It has been shown, for instance [4], that the duration of the phases of the mitotic cycle of myocytes in the auricle of the left atrium of adult rats after trauma is of the same order as in the intact myocardium of the ventricle of newborn rats. This confirms the view expressed by Sidorova [6] on similarity of the pathways of regeneration and normal ontogeny.

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EFFECT OF 5'-DEOXY-5'-S-ISOBUTYLADENOSINE ON HEMATOPOIETIC STEM CELLS

S. B. Stepanova and N. A. Fedorov

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The compound 5'-deoxy-5'-S-isobutyladenosine (SIBA), a synthetic analog of S-adenosylhomocysteine, inhibits cell transformation induced by DNA- and RNA-containing viruses and viral replication [6, 10, 11], as well as methylation of tRNA and proteins [8, 14].

Raies et al. [10] and Robert-Gero et al. [11] observed a marked decrease in transport of labeled precursors when they studied the effect of SIBA on synthesis of nucleic acids and intracellular proteins. Pierre and Robert-Gero [9] showed that SIBA depresses transport of sugars and nucleotides equally in normal and virus-infected fibroblasts.

The antimitogenic action of SIBA on blast-transformed human and animal lymphocytes has been demonstrated by several investigations [2, 3].

According to Raies et al. [10], SIBA penetrates easily into cells. Carteni et al. [4] and Lawrence et al. [7] demonstrated the rapid enzymic degradation of SIBA to the less active 5'-deoxy-5'-S-isobutylinosine in eukaryotes.

Since SIBA has a powerful oncostatic action and possesses low toxicity toward normal cells, it shows prospects of being an effective antitumor preparation. Accordingly, in the investigation described below, the effect of SIBA was studied on normal hematopoiesis and, in particular, on proliferative activity of hematopoietic stem cells (CFUs).

EXPERIMENTAL METHOD

SIBA was generously provided by the French biochemists Lederer and Robert-Gero (Institut de Chimie des Substances Naturelles, France).

Experiments were carried out on female (CBA × C57BL)F_1 mice weighing 20–22 g.

In experiments in vitro bone marrow cells were flushed out of the femora of intact mice with medium TC 199 with the addition of 20 mM HEPES and antibiotics at 4°C, and then washed out once with medium. The cell suspension (4×10^6 nucleated cells in 1 ml) intended for transplantation was incubated with SIBA (3.1×

Laboratory of Biochemistry of Leukemia, Central Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow.

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