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Cardiomyocyte Proliferation in Rat Fetuses under Normal Conditions and after Heart Injury

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Heart injury was inflicted to outbred albino rat fetuses on day 16 of prenatal development and cardiomyocyte proliferation was evaluated in the left ventricular zones adjacent to the wound and distant from it. The mitotic index of experimental animals was higher than in intact controls only for zones distant from the injury on day 10 after the intervention. The percentage of pathological metaphases gradually increased in all experimental groups, which causes doubt in the viability of postmitotic cardiomyocytes.

Key Words: *prenatal period; regeneration; cardiomyocytes; proliferation; pathological mitoses*

The problem of more favorable course of myocardial regeneration in young animals remains unclear. According to previous findings, myocardial defect in rat fetuses heals worse than in adult animals, because of slow formation of the cicatrix [1]. Since reproduction of ventricular myocytes is maximum during the prenatal ontogeny [6,7], we studied the effect of myocardial injury inflicted during this period on proliferation of these cells.

MATERIALS AND METHODS

Mechanical injury involving about 20% of total myocardial volume was inflicted to outbred albino rat fetuses on day 16 of prenatal development. The reproducibility of this injury was previously proven [1]. The animals were sacrificed by ether narcosis 1, 3, 5, 7, and 10 days after the intervention. The reference group consisted of intact animals of the same age. Histological analysis of the heart was carried out. Muscle and connective tissue cells were differentiated by the PAS reaction, carried out after amylase treatment of the sections. Cardiomyocyte mitoses were counted in the compact left-ventricular myocardium: in cardiomyocytes surrounding the injury and in the area distant from it (in intact myocardium in the control); the mitosis phase was taken into account. Pathological metaphases (chromosome dissemination, adhesion, three-group and empty metaphases) were counted specially.

Proliferation in each group was evaluated by the mitotic index (MI; expressed in percent); percent of pathological metaphases from their total number was also estimated.

Statistical analysis was carried out using Microsoft^{R} Excel 2000 and Sigma Stat 3.5 software. The phase ratio was compared using χ^2 test, the interface of 95% confidence intervals for the shares was estimated using the φ test [3]. Two selected shares were compared using z test [8].

RESULTS

Cardiomyocytes at different terms of prenatal development virtually did not differ by proliferative

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activity. Mitotic index decreased significantly only on day 2 of life (z=3.677; p<0.001) and then on day 5 (z=2.445; p=0.014; Fig. 1), which is in line with previous data indicating reduction of cardiomyocyte proliferation during the postnatal period [6,7].

Changes in proliferative activity after myocardial injury were uneven. On day 1 after the injury (day 17 of prenatal development), MI of ventricular cardiomyocytes decreased in comparison with the control (z=5.850; p<0.001 in the perifocal zone; z=6.172; p<0.001 at the periphery of the injury). On day 3, proliferation in the area adjacent to the wound and at the periphery increased (z=4.520; p<0.001 and z=6.300; p<0.001, respectively) reaching the control level, but did not surpass it. On day 5 (day 21 of prenatal development), MI in the zone adjacent to the injury decreased in comparison with the previous term (z=2.623; p=0.009) and became lower than in distant myocardial area (z=3.311; p<0.001) and in the control (z=4.112; p<0.001). On day 7 after injury (day 5 of life), proliferative activity further decreased in all analyzed zones of the left ventricle (z=2.447; p=0.013 in the perinecrotic zone; z=4.860; p<0.001 in myocardial zone distant from injury). Mitotic indexes of the perinecrotic and peripheral zones virtually did not differ, nor did they differ from the control. On day 10 after injury (day 7 of life), the direction of changes in proliferative activity changed. The MI in the area distant from the injury was higher than in the perinecrotic zone (z=2.765; p=0.006) and in intact ventricle (z=3.375; p<0.001).

Scanty and even solitary mitotic figures were often observed after mechanical injury in the postnatal myocardium inside the myofibrils around the focus of injury [6], and hence, increased mitotic activity could be expected in this zone during the prenatal period. However, even during this period no increase of cardiomyocyte proliferation in comparison with the control was noted in this area of compact left-ventricular myocardium. Myocardial injury inflicted during the fetal period, when proliferative activity of ventricular cardiomyocytes was maximum, stimulated mitotic activity of only ventricular cardiomyocytes distant from the focus of injury and only on day 10 after it was inflicted.

Proliferation was blocked during the postnatal ontogeny; the signs of blocking were prolongation of the cell cycle, *e. g.* duration of metaphases, and increased number of pathological mitoses [6]. The dynamics of mitosis phase ratio indicated changes in their duration during normal and adaptive growth.

The duration of phases of mitosis of cardiomyocyte nuclei varied during prenatal ontogeny of the myocardium until postnatal day 2, reflecting

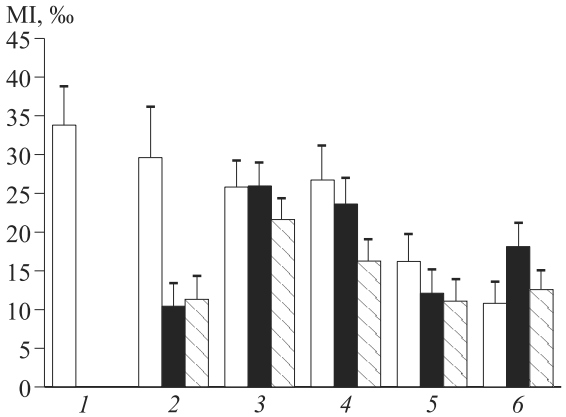

Fig. 1. Mitotic activity of cardiomyocytes after left-ventricular injury inflicted on day 16 of prenatal development. Cross-hatched bars: cardiomyocytes round the focus of injury; light bars: in areas distant from injury; dark bars: cardiomyocytes of intact control. Abscissa: numerator: age; denominator: day after injury; p.n.: prenatal development; post.n.: postnatal development. *1*) 16 p.n./0; *2*) 17 p.n./1; *3*) 19 p.n./3; *4*) 21 p.n./5; *5*) 2 post.n./7; 6) 5 post.n./10.

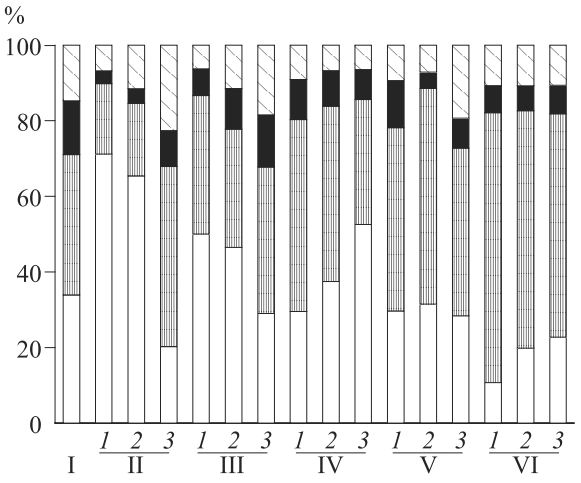

Fig. 2. Mitosis phase ratio after myocardial injury. Numerator: age; denominator: day after operation and studied heart zone: *I*) 16 p.n./0 (control); *II*) 17 p.n./1; *III*) 19 p.n./3; *IV*) 21 p.n./5; *V*) 2 post.n./7; *VI*) 5 post.n./10. Light sections of bars: prophase; vertically-hatched sections: metaphase; dark sections: anaphase; cross-hatched sections: telophase.

individual changes in the mitotic activity and its greater variability because of establishment of circadian rhythms of mitosis during this period [2] (Fig. 2). The proportion of metaphase to other mitotic phases changed negligibly, this indicating stable duration of this phase for cardiomyocytes at this stage. Though these parameters do not change on day 5 of life in comparison with day 2, a significant increase in the percentage of metaphase in comparison with that on days 16 and 21 of prenatal life (z=2.941; p=0.003 and z=3.378; p<0.001, respectively) is for the first time detected at this term,

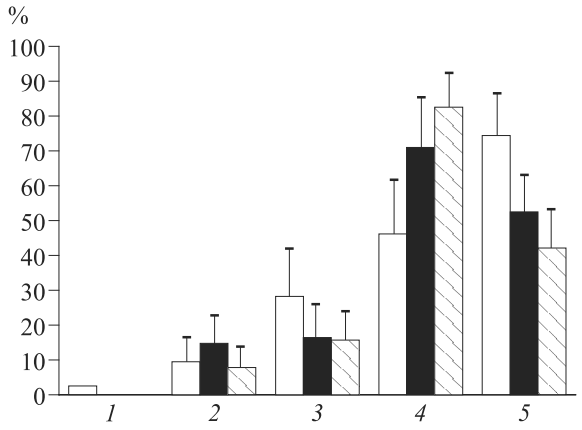

Fig. 3. Percentage of cardiomyocyte pathological metaphases and anaphases during different periods after left-ventricular injury. Light bars: cardiomyocytes of intact control of the same age; dark bars: cardiomyocytes round the focus of injury; cross-hatched bars: cardiomyocytes in an area distant from focus of injury. *1*) 17 p.n./1; *2*) 19 p.n./3; *3*) 21 p.n./5; *4*) 2 post.n./7; *5*) 5 post.n./10.

which is a result of gradual prolongation of this phase.

Drastic changes were observed in the analyzed ventricular areas on day 1 after the injury: the phase ratios were changed in comparison with the control (χ^2=45.219%; p<0.001 at 6 degrees of freedom) and of metaphase percentage (z=3.384; p<0.001 in the perifocal area and z=3.154; p=0.002 in areas distant from the focus), which indicated recovery of proliferation after its temporary suppression caused by the postoperative stress (Fig. 2). Later no appreciable differences in the ratio of phases in the perifocal and distant areas were observed, while differences in comparison with the control values were recorded only on days 19 (χ^2=35.06; p<0.001 at 6 degrees of freedom) and 21 of prenatal development (χ^2=416.115; p=0.013 at 6 degrees of freedom). No significant increase in the percentage of metaphases in comparison with the control was noted.

Hence, mitoses after heart injury in 16-day-old fetuses do not differ by phase ratio from those in the control groups, this indicating stable duration of the mitotic cycle.

However, the pattern of mitotic activity changed. Published data indicate that numerous abnormalities of mitosis are characteristic of better differentiated cardiomyocytes of postnatal stages [6]; in line with this data, we also noted increased percentage of pathological metaphases on day 21 of prenatal development (z=2.526; p=0.012) and on day 5 of postnatal development (z=2.314; p=0.021). During this period they accounted for 74% of all metaphases (Fig. 3). The increase in the number of pathological metaphases with age indicates progressive disorganization of the mitotic system, associated with reduction of proliferation.

The percentage of pathological metaphases in damaged myocardium during prenatal ontogeny did not differ from the control (Fig. 3). This parameter increased significantly on day 5 after the injury (day 2 of life: z=3.677; p<0.001) in the perinecrotic zone (z=5.084; p<0.001) and at the periphery (z=7.091; p<0.001). It is noteworthy that the parameter in the area distant from the focus was even higher than in the control (z=3.141; p=0.002). On day 10 after the operation (day 5 of life), the percentage of pathological metaphases in the area distant from the focus of injury was reduced significantly in comparison with the previous period (z=3.970; p<0.001) and control (z=3.084; p=0.002), which indicates partial normalization of mitotic processes.

The fact that a great part of cardiomyocyte mitoses was abnormal is obviously significant for the result of recovery. It was found, for example, that the cells in which DNA could be damaged or the spindle assembly disordered, could die during mitosis [4] or after transition from abnormal mitosis to G_1 phase [5]. The problem of postmitotic cardiomyocyte viability thus acquires the priority significance. Hence, not only quantitative, but also qualitative evaluation of cardiomyocyte proliferation is essential for evaluating the reparative potential of the myocardium.

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