MORPHOLOGY AND PATHOMORPHOLOGY

Death, Elimination, and Regeneration of Cardiomyocytes in Mice after Hyperthermia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 8, pp. 228-231, August, 2000
Original article submitted July 21, 2000

Single total hyperthermia changed the absolute number of cardiomyocytes in experimental animals. The total number of cardiomyocytes decreased by 20% (without signs of their necrosis) on day 3 of post-heating restitution and then returned to the control. This was probably related to cytokinesis (without karyokinesis) of binucleated cells, whose content considerably decreased during recovery.

Key Words: *total hyperthermia; cardiomyocytes; absolute number; apoptosis*

The interrelation between alteration, death, elimination, and regeneration of parenchymal cells under the effect of adverse factors determines the functional state of organs or tissues and the outcome of acute and chronic diseases. Death and elimination of parenchymal cells resulted from their alteration or plastic deficiency can lead to organ and tissue insufficiencies [4,7,16], in particular, in tissues consisting of highly differentiated parenchymal cells characterized by intracellular regeneration (*e.g.*, in the myocardium).

Despite a large body of data on cardiomyocyte (CM) alteration and regeneration of after injury, changes in the total number of parenchymal cells initiating regenerative processes at the cellular and subcellular levels received little attention. Regenerative potencies of CM and their reactions to pathological factors are poorly understood. Studies of the absolute number of parenchymal cells reflecting their elimination and regeneration will characterize regenerative processes in the myocardium.

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Here we studied changes in the total number of CM in experimental animals subjected to single total hyperthermia.

MATERIALS AND METHODS

Thirty-six male CBA mice aging 3 months were subjected to single total hyperthermia; 8 animals served as the control. The mice were placed in a temperature-controlled chamber at 43°C for 35 min. This exposure was estimated from the survival rate and corresponded to the maximum period, after which we observed total death of animals. The mice were decapitated 30 min, 3, 7, and 14 days after hyperthermia, and specimens for morphometry were taken. Control animals were kept in a vivarium under standard conditions and *ad libitum* food and water supply.

The absolute number of CM in mouse heart was estimated by the method of alkaline dissociation of fixed tissues [1,9]. As distinct from dissociation of native tissues with proteolytic enzymes or alkalis [14], this method provides complete dissociation of cells and does not change their morphological and tinctorial properties.

The heart was fixed in 10% neutral formalin for at least 10 days, and 1-mm slices were dissected from

0007-4888/00/0008-810$25.00

the right and left ventricles. The samples were weighted, placed in 50% KOH for 20-24 h, and then studied by the method described elsewhere [9].

The number of cells was estimated using a Fuchs—Rozental' counting chamber. Five chambers consisting of 2 compartments with a constant volume were filled simultaneously. The concentration of cells was calculated by the formula:

$$c=A\times V\times 10^3/3.2m,$$

where c is the number of cell in 1 mg heart, A is the mean number of cells in the compartment, 3.2×10^{-3} is the volume of the compartment (mm^3), m is the weight of the sample (mg), and V is the final volume of the suspension (ml). The absolute number of cells was calculated by the weight of the heart. All measurements were performed in 10 repetitions. The results were analyzed by Student's t test.

RESULTS

Single total hyperthermia did not change mouse body weight and weight of the heart (Table 1). Thirty minutes after hyperthermia, marked circulatory disturbances were seen in the myocardium: interstitial edema, plethora, lymphostasis, and heterogeneity of CM related to contracture-induced damages to myofibrils and cell lysis. Considerable structural and functional heterogeneity of CM was observed from day 3 after hyperthermia to the end of observations. The number of lysed CM was greater than in the former period; circulatory disturbances were retained. This period was characterized by pronounced lymphostasis. Seven days after hyperthermia, atrophic muscle fibers were replaced with small cardiosclerosis focuses. Similar changes were previously found in rat myocardium after total hyperthermia [2].

The number of CM decreased by 21% ($p<0.05$) on day 3 after heating (Table 1). This was primarily related to reduced cell concentration per 1 mg tissue (by 19%, $p<0.05$), because the weight of the heart decreased only by 5%. On days 7 and 14 after hyperthermia, the number of CM returned to normal, and their concentration increased in parallel (Table 1).

In the myocardium of control mice, binucleated CM constituted 45.3±3.9% of the total CM population, which is lower than in Wistar rats (80%) during the early postnatal period [11]. These differences were probably related to interspecies variability, peculiarities of ontogenetic periods, and various methods for studying binucleated cells. The number of binucleated CM did not considerably change 30 min after hyperthermia (Fig. 1), but progressively decreased from day 3 to 14 (to 10.7±1.7% of the total number of CM, $p<0.01$). These results suggest that restoration of the total number of CM after their death and elimination results from cytokinesis of binucleated cells (without karyokinesis). The number of CM 14 days after hyperthermia remained low despite a 4.2-fold decrease in the number of binucleated cells, which attests to ongoing death and elimination of CM.

These data indicate that extreme temperatures affect the population of CM: more than 20% CM are eliminated, but then their number returns to normal. It was shown that single food deprivation of rats followed by recovery of the body weight produces similar changes [8]. Since there were no signs of CM necrosis in mouse myocardium at all periods of restitution, their death was probably related to apoptosis or similar molecular and cellular processes.

According to current notions, apoptosis plays the major role in remodeling of organs and regulation of their cellularity under physiological and pathological conditions [13,15]. Recent studies indicate that CM death during various cardiovascular diseases (congestive heart failure, dilated and hypertrophic cardiomyopathy, infectious myocarditis, arrhythmia, atherosclerosis, and acute ischemia and reperfusion) is associated with apoptosis [10,12,16]. However, the role of programmed cell death in the regeneration of damaged myocardium, including the retainment of normal tissue architectonics, is poorly understood.

The problem of regeneration of damaged myocardium (more exactly, whether or not CM of adult mammals can proliferate) is still an open question. Autoradiography and electron microscopy showed that mammalian CM lose their ability to regenerate by mitosis at the

TABLE 1. Quantitative Analysis of CM Population in Myocardium of CBA Mice after Single Total Hyperthermia ($M\pm m$)

Parameter	Control	Time after hyperthermia			
		30 min	day 3	day 7	day 14
Weight of the heart, mg	86.2±4.4	104.4±5.1	82.0±4.1	84.5±5.7	101.2±3.5
CM content per 1 mg tissue, 10^3	19.2±1.1	18.8±1.9	15.6±0.6**	21.8±1.2	16.7±1.2
Absolute number of CM, 10^3	1647.0±88.9	1940.9±178.2	1307.3±57.6**	1863.7±211.3	1699.4±151.6
Content of binucleated cells, %	45.3±3.9	42.1±3.6	22.2±0.7*	13.6±2.5*	10.7±1.7*

Note. *$p<0.01$ and **$p<0.05$ compared to the control.

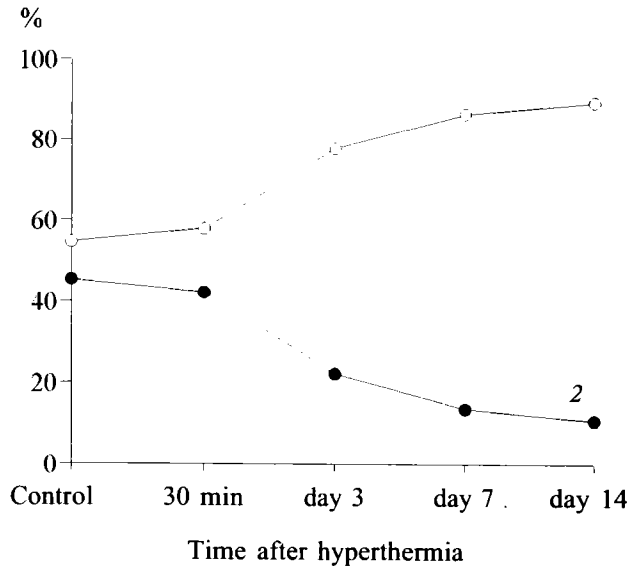

Fig. 1. Ratio between mono- (*1*) and binucleated (*2*) cardiomyocytes in mouse myocardium during recovery.

early stages of postnatal development. Furthermore, there are interspecies differences in the periods of blockade of this cell division [6,11]. In late ontogeny, regeneration of damaged myocardium proceeds via intracellular regeneration and hypertrophy of intact CM [3,7].

It should be emphasized that new experimental biological approaches are now extensively elaborated, which allow regeneration of the myocardium by induction and regeneration of CM [5]. However, these effects are short-lasting and do not provide total regeneration of damaged myocardium at the tissue level.

Hence, the absolute number of CM in rodents considerably decreases (without signs of cell necrosis) under the effect of adverse factors and returns to normal during the recovery period. The data suggest that regeneration of the myocardium at the cellular level proceeds via cytokinesis of binucleated cells without karyokinesis. This process is directed to the maintenance of the total number of CM. The CM count after their non-necrotic death and elimination is probably increased by this mechanism and continued to the exhaustion of reserve binucleated cells. Then regeneration of the myocardium proceeds via hypertrophy of CM followed by their dysfunction and development of heart failure.

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