Polyploidy of Smooth Myocytes in Coronary Arteries

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Study of smooth myocytes in coronary arteries of dogs with experimental coarctation of the aorta revealed increased DNA content in myocyte nuclei and increased percentage of binucleated cells. Polyploidy of vascular myocytes did not disappear after correction of the aortal defect.

Key Words: *vascular smooth muscle cells; polyploidy; binucleated cells*

Study of polyploid transformation of cell populations in the body, including vascular smooth myocytes (SMC) in circulatory disorders and after their removal, attracts special attention of morphologists [3,11,12]. Modeling of aortic coarctation on animals leading to arterial pressure increase in the coronary basin [2,13] and its correction are promising methods for such studies. SMC of the tunica media of coronary arteries after elimination of hypertension are little studied.

Vascular changes under conditions of hypertension and the possibility of their regression [8] are important practical problem, which became the object of our research.

MATERIALS AND METHODS

Coarctation of the aorta was created surgically [7] in experiments on eighteen 3-4-month-old puppies. After 1 year the defect was repaired in 8 of them and the animals were observed for 12 months. Material from 8 animals served as the control. Euthanasia was carried out by bleeding under narcosis. Smooth myocytes of the tunica media were isolated by alkaline dissociation [1], the preparations were stained with hematoxylin and eosin and by Feulgen's method (hydrolysis in 5 N HCl at 37°C for 12 min). The linear size of SMC and their nuclei were measured by a screw ocular micrometer, their areas and volumes were estimated [9], and the percentage of binucleated forms was evaluated. DNA content in the nuclei of mono- and binucleated SMC was analyzed on a MIF-K cytophotometer at λ=580 nm. Quantitative data were processed by the method of variation statistics.

RESULTS

In animals with experimental hypertension in the left coronary artery SMC of the tunica media and their nuclei increased were enlarged (Fig. 1, *b*) in comparison with the control (Fig. 1, *a*), which was confirmed by the results of cyto- and karyometry (Table 1). The length and width of vascular SMC increased by 1.2 and 1.5 times, respectively, the area and volume increased by 1.9 and 3.2 times, respectively. The same regularity was observed in measurements of SMC nuclei: their length and width increased by 1.1 and 1.4 times, respectively, area and volume by 1.6 and 2.2 times, respectively. Under conditions of long-term hypertension in the coronary basin SMC of the tunica media in arterial branches became hypertrophied. Cytophotometry revealed a 2-fold increase in DNA content in mononucleated SMC (Fig. 2); virtually all binucleated cells had nuclei of equal ploidy.

The percentage of binucleated SMC in the population also increased (Fig. 1, *c*, *d*) to 3.1% *vs.* 0.1% in the control (*i.e.* by 31 times). The detected changes reflect polyploid transformation of SMC in cardiac arteries under conditions of long-term hypertension.

Correction of aortic coarctation led to incomplete normalization of SMC parameters (Table 1): the

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length and width of cells decreased by 1.1 and 1.2 times, the area and volume decreased by 1.3 and 1.6 times, respectively; but the parameters did not reach the initial levels and still surpassed them 1.5- (area) and 2-fold (volume). The same trend was observed for myocyte nuclei: the length and width decreased 1.1 and 1.2 times, area and volume 1.3 and 1.6 times, respectively, but remained 1.2 and 1.3 times higher than the corresponding parameters in intact animals. Atrophy of previously hypertrophic SMC in the tunica media of coronary arteries persisted after correction of hypertension in the coronary basin. The content of DNA in the nuclei (Fig. 2) and percentage of binucleated myocytes (3%) virtually did not change.

Hypertrophy and polyploidy of SMC in the tunica media of coronary arteries developed after experimental creation of aortic coarctation. The growth of tangent tension in vessels was paralleled by activation of DNA synthesis in myocyte nuclei [6]. The increase in the number of binucleated SMC in coronary hypertension was observed for the first time in this study. This phenomenon was also detected in the cerebral arteries after modeling of aortic coarctation [9]. It is known that damaging interventions increase the number of binucleated forms in cell populations of the vascular wall [4], which is regarded as a variant of polyploid transformation [10]. We previously showed that SMC population of the tunica media is the most reactive under conditions of regeneration morphogenesis of rat aorta: the index of labeled nuclei in these cells increased 53-fold in comparison with the control [5]. Hence, under conditions of increased load (hypertension), both types of polyploid SMC formed as a result of polyploidizing mitosis (a variant of common mitosis arrested at this or that stage [10]). Our findings indicating that the ratio between DNA content in the

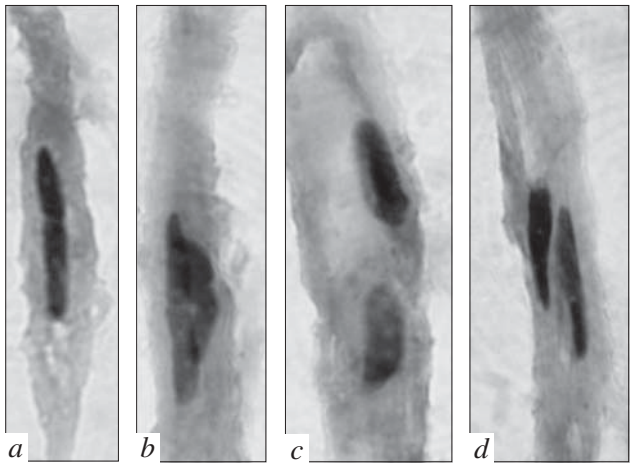

Fig. 1. Smooth myocytes of the left coronary artery in control and experiment. Alkaline dissociation. Hematoxylin and eosin staining, ×1000. *a*) vascular myocytes (control); *b*) hypertrophic nucleus; *c*, *d*) binucleated myocytes in hypertension (12-months observation).

TABLE 1. Results of Morphometry of Smooth Myocytes of the Left Coronary Artery in Control and Experiment (*M*±*m*)

Series	Cell length, μ	Cell width, μ	Length of nucleus, μ	Width of nucleus, μ	Cell area, μ^2	Cell volume, μ^3	Nucleus area, μ^2	Nucleus volume, μ^3
Control	48.5±1.5	14.1±0.5	16.8±0.3	7.5±0.2	536.0±12.8	5043.0±66.5	98.9±3.6	494.0±8.4
Arterial hypertension	57.1±1.8**	23.1±0.7**	19.2±0.4*	10.3±0.2**	1035.0±15.1*	15 935±96*	155.0±4.9**	1065.0±13.1*
After correction of arterial hypertension	52.1±1.9**	19.1±0.8*++	17.6±0.6*	8.4±0.5*+	781.0±13.1*+	9940.0±84.4*++	116.0±4.4*+	649.0±9.3*++

Note. *$p<0.01$, **$p<0.02$ compared to the control, +$p<0.01$, ++$p<0.02$ compared to arterial hypertension.

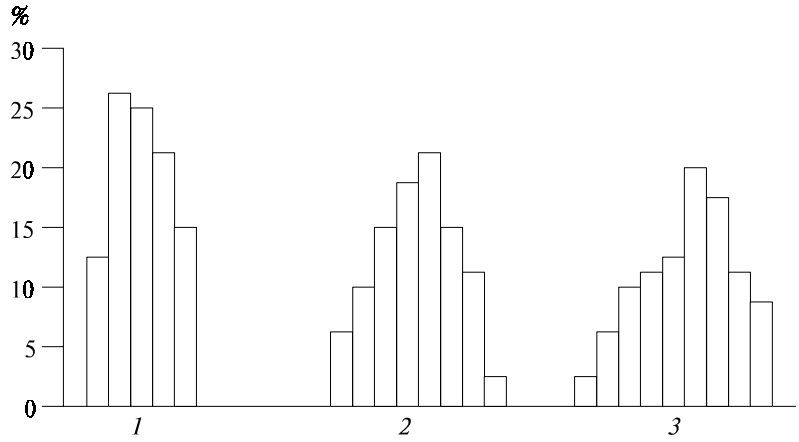

Fig. 2. Histograms of DNA content in SMC nuclei in coronary artery tunica median in dogs. Abscissa: DNA content in control (*1*; 2 c), in arterial hypertension (period of observation 12 months; *2*; 4 c), and 12 months after repair of the defects (*3*; 4 c). Ordinate: relative content of mononucleated myocytes.

nuclei of binucleated vascular myocytes is close to 1.0 also attest to the mitotic origin of binucleated elements. DNA replication provides the appearance of new cells or polyploidy of the existing cells (intensification of intracellular regeneration) [8].

Correction of aortic coarctation in animals decreased the size SMC in the tunica media, though the initial parameters were not completely restored during the studied period. The content of polyploid SMC forms after repair of artificially created defect did not change. This finding agrees with previous publication [10] and indicates that the developing polyploidy of vascular myocytes is a stable biological phenomenon.

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