CELLS CONTAINING SMOOTH MUSCLE MYOSIN DURING HEALING OF MYOCARDIAL INFARCTS IN RATS

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The first report that muscle cells take part in healing of a focus of injury in the myocardium was published by Oppel in 1901 [13]. In subsequent years investigators have repeatedly returned to these cells and have attempted to study their fate [1, 6], although doubts have still remained regarding the nature of these cells which take part in healing and whether they are indeed muscle cells [5].

The present writers showed previously [3] that in the zone of myocardial infarction myofibroblasts appear: These are cells which combine the ultrastructural features of myocytes and fibroblasts. Because they contain smooth muscle myosin, these cells can be detected immunomorphologically. However, the use of frozen sections and fluorescence labeling limited the scope for the study of the morphology of these cells and their quantitative analysis.

In the investigation described below an immunoperoxidase method was used to reveal myosin in paraffin sections. By combining this method with autoradiography, the degree of participation, zones of localization, and proliferative activity of cells containing smooth muscle myosin were determined in the pathological focus during healing of a myocardial infarct.

EXPERIMENTAL METHODS

The left coronary artery was ligated under ether anesthesia in 28 noninbred male rats weighing 120-140 g to induce myocardial infarction. The animals were killed 2, 3, 5, 7, 14, 21, 30, 60, and 120 days later. Tritiated thymidine was injected in a dose of 2.5 μCi/g body weight (specific radioactivity 12 Ci/mmole) into 12 rats 2 h before sacrifice.

The rats' hearts were fixed in 80% ethyl alcohol. Dewaxed sections 5 μ thick were treated with a 0.1% solution of pronase in buffered physiological saline (BPS: physiological saline made up of 0.01 M phosphate buffer, pH 7.2) for 5-7 min at room temperature. The sections were place in cold distilled water and incubated successively with a 1% solution of hydrogen peroxide and normal goat serum. One of two consecutive serial sections were incubated for 40 min at room temperature with rabbit antiserum against smooth-muscle myosin, the other with antiserum against striated muscle myosin. The characteristics of the antisera were described previously [7]. After washing, the sections were incubated for 30 min under the same conditions with goat antibodies against rabbit IgG, labeled with horseradish peroxidase according to the method described in [4]. The reaction was developed in medium containing 5 mg diaminobenzidine and 0.05 ml of a 3% solution of hydrogen peroxide to 10 ml BPS. Some sections were then covered with type M photographic emulsion, exposed for 30 and 45 days in a refrigerator, developed, and stained with hematoxylin or azure II and eosin.

Using Glagolev's principle [2], the relative volume of cells containing smooth-muscle myosin was determined in the focus of myocardial infarction. The central and subendocardial zones were distinguished. On histoautoradiographs in the same zones the number of labeled nuclei in connective-tissue cells and in cells containing smooth-muscle myosin was counted

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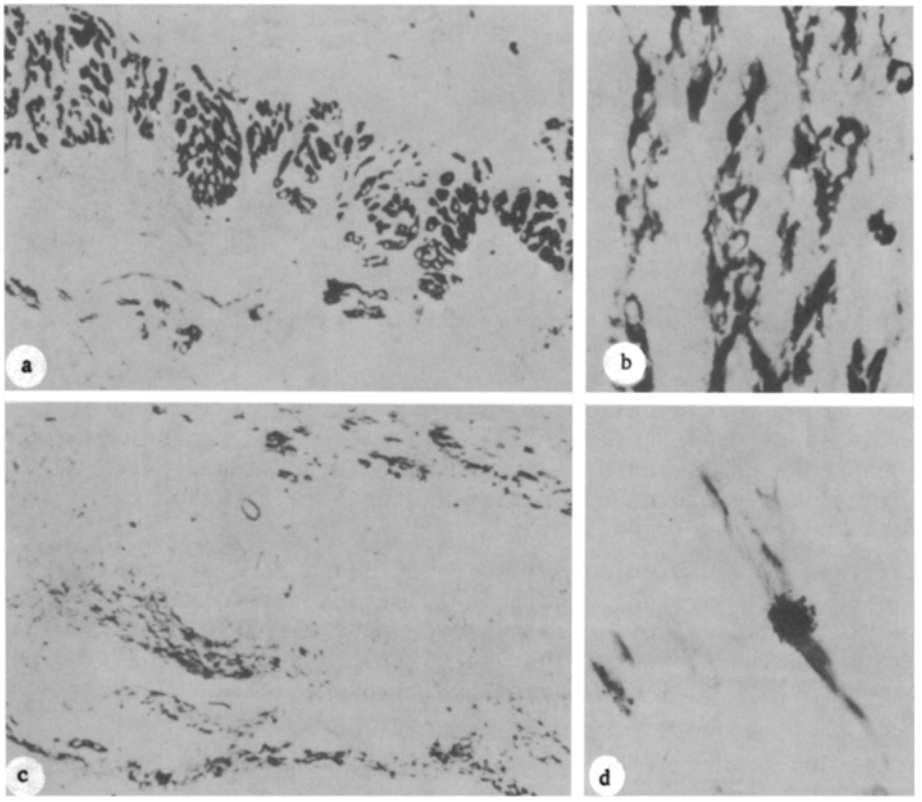

Fig. 1. Immunoperoxidase method of detecting myosin in cells in a focus of myocardial infarction. a) Residual heart muscle fibers; b) cells containing smooth-muscle myosin (on 21st day of experiment). Serial paraffin sections, 120 ×; c) group of cells containing smooth-muscle myosin in center of pathological focus on 21st day of experiment. 240 ×; d) DNA-synthesizing cell containing smooth-muscle myosin on 7th day of experiment. 630 ×. Treatment with antisera against human striated-muscle (a) and smooth-muscle (b-c) myosin with counterstaining by hematoxylin (d).

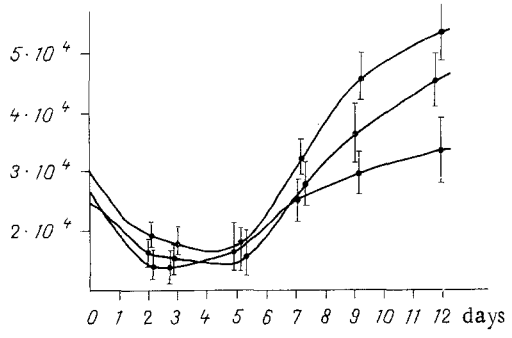

Fig. 2. Relative volume of cells containing smooth-muscle myosin in different zones of focus of myocardial infarction during healing. Abscissa, stages of experiment (in days); ordinate, relative volume (in %). Black columns indicate subendocardial zone, unshaded columns — central zone, obliquely shaded columns — mean values for whole focus on injury. [As in Russian original; caption does not match figure — Publisher.]

TABLE 1. Number of Labeled Nuclei of Connective-Tissue Cells and of Cells Containing Smooth-Muscle Myosin in Focus of Injury during Healing of Myocardial Infarct (%)

Stage of experiment, days	Central zone		Subendocardial zone	
	connective-tissue cells	cells containing smooth-muscle myosin	connective-tissue cells	cells containing smooth-muscle myosin
5	4,66±0,70	0	5,01±0,72	0,87±0,30
7	2,49±0,44	0,49±0,20	3,84±0,51	0,27±0,13
14	2,13±0,38	0,06±0,06	2,27±0,39	0,18±0,11
21	0,90±0,29	0	1,35±0,36	0,23±0,15

disregarding the myocytes of the blood vessels. The results were subjected to statistical analysis. Some sections also were stained with hematoxylin and eosin and with picrofuchsin.

An injection of 1 ml of BPS containing 5 mg of Evans' blue was given into the caudal vein of another 5 rats on the day after production of myocardial infarction and into three intact animals of the same weight. The animals were killed 2 h later and the heart was perfused and examined under a magnifying glass.

EXPERIMENTAL RESULTS

Antiserum against striated muscle myosin revealed the outlines of the pathological focus, by specifically staining the heart muscle fibers which remained a dark brown color (Fig. 1a). On treatment of a serial section with antiserum against smooth muscle myosin cells located chiefly in the subendocardial and central zones of the pathological focus were found in the zone of injury (Fig. 1b). These cells appeared on the 3rd day of myocardial infarction in the subendocardial zone and after the 5th day of the experiment in the center of the pathological focus. These cells when found beneath the endocardium differed in direction and were arranged in an interrupted narrow strip, in large concentrations in some places, and these were particularly numerous in the late stages of the experiment. Conversely, only single cells containing smooth-muscle myosin were found in the center of the pathological focus. Their direction corresponded to the circular fibers of the heart, and sometimes they were located along the vessels but without any connection with them. As a rule these cells were elongated, with a relatively small content of myosin, arranged around the periphery of the cells in the form of a rim. The nuclei of these cells often had uneven outlines and sometimes a constriction ring. The cytoplasm appeared somewhat basophilic when stained with hematoxylin, and it stained yellow with picrofuchsin. In the later stages of the experiment, three weeks after production of the myocardial infarct, these cells became more circular, they contained much more myosin, and they formed multiple junctions with each other (Fig. 1c). The relative volume of these cells was increased correspondingly, from 3.27 ± 0.24% on the 7th day to 4.84 ± 0.27% of the total volume of the pathological focus on the 21st day. At subsequent times of the experiment the relative volume of these cells decreased, although four months after the beginning of the experiment single cells containing smooth-muscle myosin could be found in the postinfarct scar.

At all times of the experiment the relative volume of cells containing smooth-muscle myosin was much greater ($P < 0.001$) in the subendocardial than in the central zone of the pathological focus (Fig. 2).

The study of histoautoradiographs stained beforehand by the immunoperoxidase method showed that the overwhelming majority of cells incorporating [^{3}H]thymidine were connective-tissue cells (Table 1). Only single labeled nuclei belonged to cells containing smooth-muscle myosin (Fig. 1d). The number of these cells was rather larger in the subendocardial than in the central zone of the pathological focus.

In rats receiving an injection of Evans' blue, a zone of intense staining with clear outlines, coinciding with the boundaries of the pathological focus, was found in the endocardium. No such picture was observed in the intact animals.

Cells containing smooth-muscle myosin thus appeared in the pathological focus on the third day of myocardial infarction and persisted in the scar during four months of the ex-

periment. By the times of appearance, ability to form contacts with each other and in their staining properties with picrofuchsin these cells were similar to so-called "myoblasts" [6]. The nuclei of cells containing smooth-muscle myosin often had uneven outlines, a characteristic feature of cells possessing contractility [10]. The unique distribution of chromatin characteristic of Anichkov's myocytes [1] was not found in them.

The contribution of cells containing smooth-muscle myosin to healing of a focus of myocardial infarction was small: Their relative volume did not exceed 5% of the total volume of the infarct. These cells were particularly numerous in the subendocardial zone, where their total relative volume for the whole period of measurement was three times greater than in the center of the pathological focus. The results of the experiment with Evans' blue go some way toward explaining this fact. We know that Evans' blue is adsorbed and stains those regions of the vascular system which do not have an endothelium intensely [8]. Staining of the endocardium in the zone of myocardial infarction is thus evidence of absence of the endothelium in this part. Consequently, the cells of this zone were accessible for blood serum proteins, whose activating role in cell proliferation is well known [14].

The proliferative activity of cells containing smooth-muscle myosin was very low, and this casts some doubt on their ability to form others of a similar kind. These cells also were located a considerable distance from one another and from their nearest neighbors. Evidently those investigators are right who suggest that myofibroblasts are formed from fibroblasts [15]. This is supported by the dynamics of the ultrastructure of these cells during healing of the myocardial infarct [3].

The role of cells containing smooth-muscle myosin in the healing of the myocardial infarct is not yet clear. The definite orientation of these cells in the center of the pathological focus, the fact that they contain a well-defined contractile apparatus — these are evidence of their functional activity, which is evidently aimed at bringing closer together the undamaged areas of the myocardium. In addition, we know that myofibroblasts synthesize type III collagen [9], which is replaced during maturation of the connective tissue by the more resistant type I collagen [12]. Under these circumstances cells containing smooth-muscle myosin are sensitive to the same pharmacological agents as vascular myocytes [11]. In this connection it is essential to discover which processes take place in the developing scar against the background of administration of spasmolytics, for the same phenomenon may take place also under clinical conditions.

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