Compensatory Hypertrophy in the Heart After Myocardial Infarction in the Rat

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Hypertrophy after myocardial infarction would be a very important process for compensation of damaged myocardium and preservation of cardiac function. Fifty-four female Sprague-Dawley rats were studied 5 weeks after randomization to infarct operation, sham operation and control groups. At sacrifice, anteroseptal infarcts ranging from 1 to 51% of left ventricle were present in the infarct operated group. When classified according to infarct size, groups with the largest infarcts (>15 to 30% and >30%) had significant (p < 0.001) cardiac cellular hypertrophy in the noninfarcted myocardium of the septum and anterior walls (fiber diameter 15.9 ± 2.3 and 14.5 ± 2.3 \( \mu m \), respectively) compared with the control group (12.0 ± 1.8 \( \mu m \)). Because of cardiac hypertrophy, remaining noninfarcted myocardial area, as estimated from serial histologic sections of the heart, was normal in the greater than 15 to 30% infarct group (area 1.35 cm\(^2\)) compared with the control group (1.43 cm\(^2\)); however, because hypertrophy plateaued in the greater than 30% infarct group, myocardial area was significantly decreased (1.06 cm\(^2\), p < 0.001), but was still more than expected without hypertrophy.

We suggest that hypertrophy accompanies large infarction in the rat and is a compensation for preserving tissue volume lost by infarction. This compensatory response appears to have limitations, such that when very large amounts of myocardium become necrotic, there is not enough hypertrophy to return myocardial volume to normal.

After acute myocardial infarction, early and long-term prognosis of patients is highly dependent on the amount of remaining viable myocardium. Cardiac hypertrophy, a non-specific compensatory mechanism for increasing mass and thereby functional capacity, would be a beneficial response after myocardial infarction. Despite the clinical prevalence of infarction, little is known about the subsequent development of hypertrophy (1). Does hypertrophy occur after infarction, and if so, to what degree? Is there a relation between the size of the infarction and the degree of hypertrophy? Unfortunately, in the clinical setting, in addition to other limitations of autopsy studies, patients who die with coronary disease usually have coexisting stimuli for hypertrophy, such as arterial hypertension, valvular dysfunction and dyskinetic wall motion (aneurysm). Thus, the process of hypertrophy in relation to myocardial infarction is difficult to study in human beings.

The rat readily develops cardiac hypertrophy under a variety of loading conditions and disease states (2); therefore, it should be a suitable model for the study of the development of hypertrophy after myocardial infarction in attempts to answer the questions posed.

Methods

Animals. Sprague-Dawley female rats (Hilltop Laboratories), with an initial weight of approximately 140 g (7 to 8 weeks old), were housed with adequate cage space proportional to body weight, subjected to 12 hour light/dark cycles and permitted water and rat chow as desired.

Method of infarction. The rat has a single left coronary artery that branches into many subdivisions at the high anterior base of the left ventricle adjacent to the right ventricular outflow tract (3). We modified a procedure for infarction that has been previously described (4). The rat was placed under ether anesthesia, the chest incised and the chest cavity entered through the fourth intercostal space. The left coronary artery was not usually visible on the cardiac sur-
face, but the visual impression of a groove between the left atrial appendage and right ventricular outflow tract formed a landmark for its location. In order to visualize this area adequately, it was sometimes necessary to retract the thymic fat pad rostrally. The heart was left in situ and a burn was made into the myocardium using a miniature soldering iron (Antex) with a 1/16 inch (0.16 cm) spade tip. The burn produces thermal injury discretely localized to the anterior basal segment. Myocardial infarction occurs distal to this point as a result of thrombotic interruption of the coronary artery. The chest was closed in two layers—first ribs and then chest wall muscle/skin—with 2-0 silk sutures. Sham group animals underwent operation identical to that of infarct group animals except no burn was made in the myocardium. Control animals did not undergo any operative or anesthetic procedure. The infarct produced by this technique is apico-anterolateral and always spares the septal wall and the majority of the posterior wall of the left ventricle (3).

**Assignment of rats to groups.** Of a total of 49 rats operated on to produce infarction, 15 died, all within 24 hours after operation, and most within the few minutes after chest closure. Death was usually attributed to massive mechanical dysfunction associated with infarction, but in some cases respiratory arrest associated with anesthesia was considered a contributing cause. Of 11 sham operated rats, 2 died immediately postoperatively. Ten rats not operated on were assigned to the control group. Health and vigor were considered a contributing cause. Of 11 sham operated rats, 2 died immediately postoperatively. Ten rats not operated on were assigned to the control group. Health and vigor were observed in all animals before operation and were also observed in all survivors 72 hours after operation. All operative wounds eventually healed well. Animals were kept for exactly 5 weeks after operation and then were sacrificed by ether overdose.

**Histologic Techniques**

After sacrifice, the ventricles were dissected free of pericardium and soft tissue at the base of the heart and gently blotted dry. The right ventricular free wall was dissected off of the left ventricle and the ventricles were weighed on an analytical balance. The left ventricular cavity was gently stuffed with cotton to preserve morphologic features and was fixed in 10% neutral buffered formalin. After fixation, each heart was cut from apex to base into four transverse slices. After processing, two serial histologic sections were obtained from each slice. It is recognized that the standard methods of tissue processing for light microscopy result in substantial shrinkage and, therefore, uniform underestimation of tissue size. Nevertheless, comparative morphologic measurements between animals and across groups remain valid. One set of sections was stained by Masson’s trichrome method for demonstration of fibrous tissue, and the other set was stained with hematoxylin and eosin for cell morphology.

**Estimation of infarct size.** Infarct size was expressed as percent of left ventricle as determined by planimetry of projections of the heart slices at about 10 × magnification. The ratio of scar length to total circumference (Fig. 1) of each section was determined by averaging epicardial and endocardial total length and scar length measurements (3,5). Infarct size was determined as a length-normalized average of the four slices. Area measurements of infarct size were not used because these have been shown to underestimate infarct size because of resorption of necrotic tissue and subsequent wall thinning (5). The validity of this length method for measurement of infarct size may also be understood by consideration of the principles of stereology, in which a three-dimensional object (for example, left ventricle) is decomposed into a series of parallel planes (for example, myocardial sections). Measurement of length in the parallel planes, and summation of these lengths, approximates the surface area of the object.

**Estimation of ventricular hypertrophy.** We used three methods to estimate the degree of left ventricular hypertrophy. First, the hearts were weighed as previously mentioned. Second, the noninfarcted myocardial area in each heart slice, which would reflect hypertrophic changes present, was measured by planimetry under magnification and the area was expressed as the sum of the four slices. Third, the fiber diameter was also measured in the ventricular septum using a calibrated microscope eyepiece grid to confirm that any increase in ventricular mass observed was due to myocardial cell hypertrophy. Twenty longitudinally-oriented fibers were measured sequentially across the septum from left to right in slice two (midventricle). We used these measurements to obtain an average of septal fiber diameter. The coefficient of variation of fiber diameter (standard deviation of diameter divided by mean of fiber diameter) was similar for the sample of 20 fibers in each animal and averaged about 10%. In addition to the septum, fiber diameter was also measured in the same midventricular section, but this time in the anterior wall adjacent to, but not directly involved by, the fibrous infarction scar. These additional measurements were made in subgroups of animals with large infarction.

All morphologic measurements were made without exact knowledge of infarct size. It was not possible to blind the observer to the presence or absence of infarction. Interobserver variability of measurements of infarct size and remaining myocardial area was less than 10% of the measured value as was intraobserver variability of measurements of infarct size, remaining myocardial area and fiber diameter.

**Statistical analysis.** On the basis of statistical sampling theory, a sample of 20 fibers in each rat was measured for diameter, and averaged to obtain the estimate of mean fiber diameter. This sample size satisfies the parameters of probability such that the sample mean will be within 1 standard deviation of the true mean at the 95% confidence level (6).

The effect of infarction was examined both by treating infarction as a continuous variable and by classifying ani-
mals by size of infarct (control, sham, 0 to 15%, > 15 to 30%, > 30%). Even though initial weight, final weight and change in weight were not different among the rat groups, we corrected our data for subtle variations of weight within a group by an analysis of covariance (7). This technique uses both regression analysis to correct for rat weight and analysis of variance to compare data between groups of rats. When a variable was found to be statistically significant by this method of analysis, individual groups were subjected to a multiple comparison test (Scheffe test) to determine which groups caused statistical significance. In addition, simple regression analysis was employed when infarct size was treated as a continuous variable. Analysis of covariance was used to determine the relation between noninfarcted myocardial area and fiber diameter across the groups. Values of variance are expressed as mean ± standard deviation. Statistical significance was set at the 0.05 level (p ≤ 0.05).

Figure 1. Myocardial hypertrophy associated with infarction. a. Tissue section of noninfarcted heart at midventricle. b. Tissue section of heart with healed infarct (arrows) from an animal in the greater than 15 to 30% infarct group (a and b, septum to the right and anterior wall at top). c, Myocardial fibers from heart shown in a, d, Markedly hypertrophied myocardial fibers from heart shown in b (c and d, trichrome stain). In both photomicrographs, which are at identical magnification, the bar equals 20 μ. In b, the epicardial and endocardial scar lengths are demarcated by arrows for this slice. Infarct size was determined as the quotient of the sum of the scar lengths in all four slices divided by the sum of the total lengths. See text for details.

Results

Body weight. At the time of randomization into groups, rat weight was 144 ± 11 g (mean ± standard deviation) and was not significantly different among the groups (p >
Five weeks later at autopsy, rat weight was 254 ± 21 g and was not significantly different among the groups (p > 0.2). Growth between operation and autopsy, as measured by weight change, was not different among the groups (p > 0.2). Clinically, the rats were indistinguishable at the time of sacrifice. Gross autopsy showed findings confined to the heart and pericardium. Neither free pleural fluid nor free peritoneal fluid was observed in any of the animals.

**Infarct size.** Neither control nor sham group animals demonstrated any evidence of myocardial infarction. Animals in the 0 to 15% infarct group had an average infarction of 6 ± 5% of left ventricle. These infarcts usually comprised epicardial burns, occasionally surrounded by small areas of infarction. Anatomic study of the infarcts in the groups with the largest infarcts showed infarction in the apicoanterolateral region, adjacent to the infarction in the two groups of animals with infarction. Therefore, we estimated that the burn itself resulted in a low percent of infarction. Animals in the greater than 15 to 30% infarct group had an average infarct size of 23 ± 4%, and those in the greater than 30% infarction group had an infarct size of 38 ± 7%. These later two groups showed infarction in the apicoanterolateral region, remote from the area of burn. There were areas of transmural infarction and subendocardial and subepicardial infarction.

**Infarct histology: fiber hypertrophy in septum.** Histologic study of the infarcts in the groups with the largest infarcts showed severe wall thinning and almost complete replacement of muscle by fibrous tissue. Microscopically, the septum was always spared. In the septum, the myocardial fibers were normally organized. There was no evidence of intramyocardial edema or fibrosis. In some groups, there was evidence of fiber hypertrophy (Fig. 1 and 2). Fiber diameters averaged 12.0 ± 1.8 μm in the control group, 11.6 ± 1.9 μm in the sham group, 11.6 ± 1.5 μm in the 0 to 15% infarct group, 15.9 ± 2.3 μm in the greater than 15 to 30% group and 14.5 ± 2.3 μm in the greater than 30% group and were significantly increased in size in these latter two groups (p < 0.001). Fiber diameter was significantly correlated with infarct size (r = 0.50; p < 0.01). It was also correlated with septal thickness in all groups (r = 0.70).

Fiber diameter was also measured in the anterior wall adjacent to the infarction in the two groups of animals with infarct size greater than 15%. In the greater than 15 to 30% infarct group, anterior fiber diameter was 15.6 ± 1.4 μm, and in the greater than 30% infarct group it was 15.0 ± 1.4 μm (p > 0.2, septal compared with anterior wall fiber diameter for both groups). Thus, the anterior wall fibers were hypertrophied to the same extent as the septal fibers. Even though the degree of hypertrophy appeared to be more severe in the greater than 15 to 30% infarct group than in the greater than 30% group, there was no statistical significance between the two groups in either the septum or anterior wall (p > 0.2).

**Fiber hypertrophy in remaining myocardial area.** Remaining, noninfarcted myocardial area was decreased about 25% in the greater than 30% infarct group, compared with that in the control group (1.06 versus 1.43 cm², p < 0.002) but was not decreased in the greater than 15 to 30% infarct group compared with control (1.35 versus 1.43 cm²; p > 0.20). Myocardial area was modestly, but significantly, inversely correlated with infarct size (r = −0.55; p < 0.01). When the noninfarcted area was compared with fiber diameter, there was a significant linear relation (p < 0.001, Fig. 3). However, the groups were significantly different from each other because of increases in the fiber diameter in greater than 15 to 30% and 30% or greater infarct groups and decreases in area in the 30% or greater infarct group (p < 0.001). Therefore, remaining myocardial area is a function of both the amount of myocardium remaining after infarction and the degree of fiber hypertrophy. Despite substantial loss of myocardium due to infarction, noninfarcted myocardial area in the greater than 15 to 30% infarct group was equal to that of control hearts because of fiber hypertrophy. Because fiber hypertrophy did not increase further in the 30% or greater infarct group, area was decreased, but still more than would be expected without hypertrophy.

**Heart weight.** Heart weight was measured in 37 of the animals and was increased 16% in the 30% or greater infarct group compared with the other groups (0.97 versus 0.84 g, p < 0.004). Figure 4 shows that both left and right ventricular weight were increased. Heart weight was modestly, but significantly correlated with infarct size (r = 0.43; p < 0.05).

**Discussion**

**Previous studies of rat infarction.** Rat myocardial infarction has been studied for about 30 years. Those previous studies showed that rat myocardium quickly undergoes co-

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<tr>
<td>Sham</td>
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*Statistically significant difference from values in control and sham groups, p < 0.001.*
agulation necrosis, inflammation, wall thinning and healing by fibrous scar formation (5). This process is usually complete by 3 weeks after infarction. Relatively little attention has been paid to the morphology of cardiac tissue not involved in the infarction, or to the effect of time on morphology chronically after infarction. On the basis of heart weight, it has been suggested that hypertrophy occurs in rat hearts 12 weeks but not 6 weeks after infarction (8). However, that study did not include microscopic examination of the heart and did not take into account that cardiac tissue is lost as a result of resorption of necrotic myocardium during healing; thus, heart weight alone would underestimate the degree of hypertrophy. Subsequently, it was observed that 4 weeks after rat infarction both hypertrophic and atrophic cells could be found at the edge of the infarct (9). However, no attempt to quantitate these changes was made.

In the first report (10), to our knowledge, of cardiac fiber measurements, cellular hypertrophy was observed in both left and right ventricular free walls 4.5 weeks after infarction. However, only qualitative statements were made about infarct size and no correlative measurements of cell size and remaining myocardial area were made. A more recent preliminary report (11) demonstrated that after infarction there was increased fiber diameter in papillary muscles.

**Findings of current study.** In this study, we observed that 5 weeks after myocardial infarction, hypertrophy occurs in areas remote from infarction. Rats with infarction greater than 15% had considerable myocardial hypertrophy. The degree of hypertrophy appeared to correlate with the size of the infarction. The correlation was modest because fiber diameter appeared to peak in the greater than 15 to 30% group, with no additional increase in the 30% or greater group. Even if fiber hypertrophy occurs in width only, an increase in diameter from 12 to 15 μm means that fiber area will increase 25% and fiber volume will increase more than 50%. It is of interest that in spite of the limitations of measuring fiber size in routine histologic sections, the calculations of fiber area appear to agree with our gross morphologic measurements of remaining noninfarcted myocardial area.

In animals with up to 30% infarction, hypertrophy appeared to keep pace with myocardial loss from the infarction so that remaining noninfarcted myocardial area was equal for control, sham, 0 to 15% and greater than 15 to 30% infarction groups. However, hypertrophy did not increase further in the greater than 30% infarct group so that remaining noninfarcted myocardial area was less than in control, sham or small infarct groups. In this group, it appears that hypertrophy is unable to keep pace with myocardial loss from infarction, and suggests that there may be a limit to the degree of compensatory hypertrophy.

**Role of hypertrophy in preservation of myocardial performance.** Perhaps this limitation of hypertrophy also
limits the functional capacity reserve of the myocardium after infarction. Studies of cardiac performance in rats 3.5 weeks after infarction have suggested that indexes of systolic function are especially decreased only in rats with greater than 30% infarction (12,13). In dogs with “nonmassive” infarction (not defined by the authors [14]), indexes of systolic function were not decreased at 3.5 and 7 weeks after infarction. We suggest that hypertrophy associated with infarct size less than 30% preserves contractile function in rats and dogs. This hypothesis assumes that hypertrophied muscle has normal contractile function. In fact, preservation of contractile function of hypertrophied rat papillary muscle after infarction has been reported (11). Therefore, studies of systolic function after infarction may need to assess the degree and physiologic significance of accompanying hypertrophy as part of conclusions concerning cardiac performance, especially if hypertrophy is variable.

Similarly, design and interpretation of diastolic compliance (pressure-volume relation) studies may need to consider the role of hypertrophy after infarction. Considerable debate exists as to whether observed changes in diastolic compliance after myocardial infarction are entirely due to the infarct region itself. A recent study of compliance in the rat (12) suggests that infarct tissue alone could account for changes in compliance—that is, the fibrous aneurysm causes an increase in unstressed diastolic volume without a change in the stiffness constant. An overall increase in connective tissue throughout the heart could change compliance, but fibrosis appears confined to the area of infarction (10). However, the presence of hypertrophy may require consideration of additional factors in the modeling of compliance to satisfy the anatomic accompaniments of myocardial infarction.

Right ventricular hypertrophy. Our data suggest the coexistent development of right ventricular hypertrophy in rats with large left ventricular infarction, based on our observations of increased right ventricular weight. This issue was directly addressed in a study (10) that observed an increase in cell size of the right ventricular free wall and coexistent medial hypertrophy of the muscular pulmonary arteries. The authors suggested that pulmonary hypertension might accompany left ventricular infarction, and more recent measurements of right ventricular pressure (13) have shown this to be true in rats with a large left ventricular infarct.

Concepts in estimation of hypertrophy. Conceptually, statements concerning hypertrophy in any cardiac disease (but especially in infarction) require the separation of myocardial tissue from other tissue types. Heart weight measurements may overestimate hypertrophy if edema or interstitial fibrosis is present, or if there is a large mass of dense infarct scar. In contrast, heart weight measurements may underestimate hypertrophy when extensive resorption of necrotic myocardium with wall thinning occurs in a large infarction. Wall thickness measurements may have similar objections and are further compromised when cavity dilation occurs. Myocardial area or volume measurements have similar limitations. Protein concentration studies require the separation of connective tissue proteins, enzymatic proteins and myofibrillar proteins to demonstrate hypertrophy. Even fiber diameter measurements may not provide an exact representation of hypertrophy if there are changes in fiber length, fiber number or variations of fiber geometry.

Clinical implications. Inconclusive evidence obtained from autopsy and cardiac catheterization suggests that hypertrophy occurs in human hearts as a sequel to infarction. Serial measurements by echocardiography appear to offer the opportunity to study the development of the hypertrophic process after myocardial infarction (15,16), and to observe the effects of hypertrophy on systolic and diastolic function of the left ventricle and its possible prognostic significance for morbidity and mortality. Furthermore, the pathogenesis of hypertrophy after infarction is not well understood (1). On the basis of our identification of myocardial hypertrophy in the rat after infarction, additional studies are suggested to define the stimuli and modifying influences of hypertrophy, to determine the functional cardiac effect accompanying the hypertrophy and to more precisely define the degree of hypertrophy and distribution of the hypertrophied fibers in the noninfarcted myocardium.

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