Progressive Ventricular Remodeling in Response to Diffuse Isoproterenol-Induced Myocardial Necrosis in Rats

John R. Teerlink, Janice M. Pfeffer, Marc A. Pfeffer

Abstract The purpose of the present study was to gain a better understanding of the relation between ventricular remodeling and heart failure by assessing the adaptation of the heart through time to graded myocardial injury in the presence of a patent coronary circulation. Left ventricular (LV) remodeling is a dynamic response of the heart to injury and a critical component in the development of heart failure. However, most previous studies have been in the presence of an occluded coronary vessel, which may in itself effect remodeling. Male Wistar rats received two subcutaneous injections of either 0, 85, 170, or 340 mg isoproterenol per kilogram of body weight. At 2, 6, and 16 weeks after injection, LV pressure, the pressure-volume relation, and histology were assessed. The graded myocardial necrosis produced in isoproterenol-treated rats was associated with dose-dependent increases in LV end-diastolic pressure, volume indexes, and global diastolic wall stress. In the higher dose groups, the LV continued to enlarge after 2 weeks, resulting in a further reduction in the ratio of LV mass to volume and a persistent rise in diastolic wall stress. These progressive changes in LV structure were associated with an increase in long-term mortality in rats from the intermediate- and high-isoproterenol dose groups. The present study in rats demonstrates that diffuse isoproterenol-induced myocardial necrosis results in a progressive enlargement of the LV cavity that is out of proportion to mass, a finding similar to that observed in discrete myocardial infarction. (Circ Res. 1994;75:105-113.)

Key Words • heart failure • pressure-volume relation • left ventricular volumes • left ventricular wall stress

Left ventricular (LV) remodeling is a dynamic response of the heart to alterations in work load and damage and is a major component in the development of heart failure. The resulting ventricular enlargement has been shown to be an important predictor of mortality in both myocardial infarction and heart failure patients. The process of ventricular remodeling has been studied in humans and many animal models, predominantly in cases with a segmental loss of myocardium through coronary occlusion and resultant myocardial infarction. These studies of discrete damage demonstrated that the initial loss of myocardium can initiate a progressive process of global ventricular enlargement. However, in most of these studies, ventricular remodeling is studied in the context of a compromised coronary artery circulation or ongoing myocardial damage (as in the Syrian hamster cardiomyopathy or viral myocarditis models). The increasing effectiveness of reperfusion therapy and the potential confounding effects of an abnormal blood supply make an understanding of ventricular remodeling in the presence of a patent vasculature essential.

The initial investigations of Rona et al demonstrated that the subcutaneous administration of isoproterenol (ISO) in rats produced graded myocardial necrosis, ranging from patchy subendocardial necrosis to transmural infarction, while maintaining a patent coronary vasculature. Beznak and Hacker demonstrated that the rat model of ISO-induced myocardial necrosis also resulted in a dose-related reduction in the functional capacity of the heart. We hypothesized that the process of remodeling in a ventricle with diffuse myocardial necrosis and a patent coronary circulation would also be progressive and similar to that observed in one with a discrete myocardial infarction due to coronary artery ligation. Our purpose was to gain a better understanding of the relation between ventricular remodeling and heart failure by assessing the adaptation of the heart through time to remote and graded myocardial injury produced by ISO in the presence of a patent coronary circulation.

Materials and Methods

General

Normotensive 13- to 15-week old male Wistar rats (Viral-free, Charles River Laboratories) were randomly selected to receive two subcutaneous injections (separated by a 24-hour interval) of 0, 85, 170, or 340 mg DL-ISO hydrochloride (Sigma Chemical Co) per kilogram of body weight. ISO solutions were prepared with sterile distilled water immediately before injection and were used within 45 minutes of preparation. Body weights were obtained before injection and hemodynamic study. Animals were housed in plastic cages and provided rat chow and water ad libitum. Experimental studies and handling of the animals conformed to the “Position of the American Heart Association on Research Animal Use” adopted November 1984 by the American Heart Association.
At 2, 6, and 16 weeks after injection, animals from each of the four dosage groups were randomly selected for study.

**LV Pressures and Pressure-Volume Curves**

Two, 6, and 16 weeks after completion of the injection protocol, hemodynamic studies were performed by methods previously described in detail. After ether anesthetic induction, a tracheostomy was performed, and ventilation and anesthesia were maintained by a positive-pressure respirator connected in series to an ether-drip apparatus. The left carotid artery was cannulated with a saline-filled catheter attached to a pressure transducer (Millar Instruments) and advanced into the LV, where measurements of phasic and mean ventricular pressures, including the LV end-diastolic pressure (LVEDP), were obtained under light anesthesia and spontaneous respiration. A midsternal thoracotomy was then performed by heat cauternizer, and the ascending aorta was exposed through blunt dissection. The heart was arrested in diastole with potassium chloride, and a double-lumen catheter was inserted via the aorta into the LV cavity, where it was secured by a ligature around the atrioventricular groove. The right ventricle was incised to eliminate any compressive effects on the LV. Within 10 minutes of cardiac arrest and before the onset of rigor mortis, at least two reproducible pressure-volume curves were generated over a pressure range of 0 to 30 mm Hg by the continuous recording of ventricular pressure during the infusion of saline at a constant rate of 0.74 mL/min. The LV volume was determined at every millimeter of mercury of pressure on the pressure-volume curve and was used to give an index of volumes at in vivo ventricular pressures. Three ventricular pressures were selected to determine LV volumes from these pressure-volume curves: the LVEDP obtained at rest at the beginning of the study was used as an index of the baseline operating volume, and pressures of 5 and 20 mm Hg were used to permit comparisons between ventricles at a common low and high filling pressure, respectively. All volumes were indexed for body weight (in milliliters per kilogram).

The passive pressure–volume relation was analyzed at five pressure ranges with curve-fitting functions. From 0 to 3 mm Hg, the pressure-volume relation was linear, and the curve-fitting expression was \[ P = k_1 V + b, \]

where \( P \) is the measured pressure (in millimeters of mercury), \( V \) is the chamber stiffness constant for this pressure range, and \( b \) is the LV chamber volume (in milliliters). Above 3 mm Hg, the pressure-volume relation was exponential, and the curve-fitting function, \( P = e^{cV} \), was used to derive the constants for specific pressure ranges: \( k_3 \) (3 to 30 mm Hg), the overall chamber stiffness constant; \( k_{10} \) (3 to 10 mm Hg); \( k_{20} \) (10 to 20 mm Hg); and \( k_{30} \) (20 to 30 mm Hg). This form of analysis permits the comparison of animals at different times and from different treatment groups. Analysis of global ventricular wall stress was performed by use of volume measurements from the pressure-volume relation and a spherical model for ventricular geometry. Diastolic wall stresses (\( \sigma_d \)) were derived from the following formula: \( \sigma_d = P/(a^2 - a^2) \), where \( P \) is LVEDP (in millimeters of mercury), \( a \) is the internal radius, measured as \( (4/3 \times \text{ventricular volume})^{1/3} \), and \( b \) is outer radius, measured as \( [\text{ventricular volume} + (1.05 \text{ mL/g} \times \text{ventricular mass})]^{1/3} \). After obtaining the pressure-volume curves, the LV was then fixed at the volume that corresponded to 5 mm Hg distending pressure and immersed in formalin for at least 24 hours.

**Pathological Studies**

The lungs were examined for thoracic infections, and a general survey of internal organs for areas of necrosis was performed; kidney weights were also obtained. The atria and great vessels were trimmed from the ventricles, the right ventricular free wall was dissected from the LV, and both ventricles were cut separately. The LV was dehydrated in alcohol, cleared in xylene, and embedded in paraffin; 50-μm-thick sections were cut serially from base to apex. From 10 to 17 (mean, 13) slices at 1-mm intervals were stained with Masson’s trichrome from which hematoxylin was omitted. The serial sections were mounted on slides and projected at a magnification of 10. A pathology grade (modified from Rona et al.) was assigned to each slice as follows: 0, no damage; 1, patchy fibrosis in one to five areas (<20% of the field); 2, patchy fibrosis in more than five areas (≥20% of the field); 3, contiguous subendocardial fibrosis of less than half the circumference; 4, contiguous subendocardial fibrosis of more than half the circumference; 5, transmural fibrosis of less than half the circumference; 6, transmural fibrosis of more than half the circumference; and 7, total transmural fibrosis with thickened scarring of more than half the circumference and no residual muscle. These grades for individual slices were summed and then divided by the number of slices from base to apex to produce a single average pathology score for the entire ventricle. Scoring was done on coded samples by an independent observer blinded to dose and time duration.

**Statistical Analysis**

Results are expressed as mean±SEM indexed for body weight, except where otherwise indicated. Twelve groups based on the four doses of ISO (0, 85, 170, and 340 mg/kg) and the three study periods after injection (2, 6, and 16 weeks) were compared. All statistical analysis was performed with the STATVIEW (version 4.01) and SUPERANOVA (version 1.11) statistical packages (Abacus Concepts, Inc.). A two-factor (time and dose) ANOVA was conducted to determine whether there was a significant interaction effect between dose and time for each variable, with predetermined means-comparison contrasts adjusted for multiple comparisons to test for differences between specific groups. If there was no significant interaction between dose and time as well as no significant effect of time for a particular variable, its values for each dose were pooled, and a mean was calculated, which is presented in the text. χ² analysis of mortality was also performed on data from the 6- to 16-week time interval for each dose group compared with the control group. A level of \( P < .05 \) was selected as representing a statistically significant difference in all of the above analyses.

**Results**

**Physical and Hemodynamic Characteristics**

A total of 150 rats underwent measurements of LV pressure and morphological and pathological variables, with roughly equal numbers of animals within each time and dosage group. At the time of baseline, there were no statistically significant differences in any of the groups for age (99±1 days) or body weight (400±3 g) and no differences in age or body weight at the terminal study within each time period: at 2 weeks (117±2 days, 443±6 g); at 6 weeks (139±1 days, 476±6 g); and at 16 weeks (210±2 days, 568±7 g). Although there was a progressive increase in body weight with aging (see Table 1; 400 to 568 g; \( P < .0001 \)), there was no effect of ISO on growth. The kidney weight–to–body weight ratio, an index of normal growth, was not significantly different with respect to either time or dose of ISO (Table 1).

The overall LV pathology score for the control rats was 0.0±0.0, although at week 16 the score of 0.1±0.1 reflected minor amounts of fibrosis near the atrioventricular junction and at the base of the papillary muscles. At all time periods, the scores for the other dose groups were statistically different not only from the control rats (Table 1), but more important, they were all statistically different from each other (\( P < .001 \)). The pathology score demonstrated the dose-related graded fibrosis produced in these rats: 0.0±0.0 (control group),...
TABLE 1. Selected Variables by Time and Isoproterenol Dose

<table>
<thead>
<tr>
<th>ISO Dose Group</th>
<th>Time</th>
<th>n</th>
<th>BW, g</th>
<th>KW/BW, g/kg</th>
<th>LV Path Score</th>
<th>LVEDP, mm Hg</th>
<th>LV Mass Index, g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg/kg</td>
<td>Week 2</td>
<td>11</td>
<td>463±10</td>
<td>8.4±0.3</td>
<td>0.0±0.0</td>
<td>2.5±0.3</td>
<td>1.88±0.04</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>15</td>
<td>490±12</td>
<td>8.0±0.2</td>
<td>0.0±0.0</td>
<td>2.3±0.2</td>
<td>1.85±0.03</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>15</td>
<td>575±13</td>
<td>8.0±0.2</td>
<td>0.1±0.1</td>
<td>2.6±0.2</td>
<td>1.82±0.02</td>
</tr>
<tr>
<td>85 mg/kg</td>
<td>Week 2</td>
<td>11</td>
<td>440±11</td>
<td>8.1±0.2</td>
<td>2.0±0.2</td>
<td>3.5±0.4</td>
<td>1.99±0.04</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>11</td>
<td>466±14</td>
<td>8.2±0.3</td>
<td>1.9±0.3</td>
<td>3.7±0.4</td>
<td>1.98±0.06</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>15</td>
<td>551±14</td>
<td>7.9±0.2</td>
<td>1.8±0.2</td>
<td>3.8±0.6</td>
<td>1.96±0.05</td>
</tr>
<tr>
<td>170 mg/kg</td>
<td>Week 2</td>
<td>11</td>
<td>450±10</td>
<td>7.8±0.2</td>
<td>2.8±0.3</td>
<td>7.2±0.9</td>
<td>2.03±0.05</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>12</td>
<td>457±10</td>
<td>7.9±0.2</td>
<td>2.8±0.3</td>
<td>6.8±0.6</td>
<td>2.04±0.04</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>14</td>
<td>573±15</td>
<td>7.7±0.2</td>
<td>2.7±0.3</td>
<td>7.1±0.8</td>
<td>2.03±0.05</td>
</tr>
<tr>
<td>340 mg/kg</td>
<td>Week 2</td>
<td>12</td>
<td>421±14</td>
<td>8.1±0.3</td>
<td>3.6±0.2</td>
<td>10.3±1.0</td>
<td>2.11±0.07</td>
</tr>
<tr>
<td></td>
<td>Week 6</td>
<td>10</td>
<td>488±10</td>
<td>7.8±0.3</td>
<td>3.9±0.2</td>
<td>9.9±0.9</td>
<td>2.14±0.04</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>14</td>
<td>574±14</td>
<td>7.8±0.2</td>
<td>3.8±0.2</td>
<td>10.1±1.3</td>
<td>2.11±0.04</td>
</tr>
</tbody>
</table>

P value

| Interaction effect | .2744 | .7399 | .7677 | .8269 | .9879 |
| Time effect        | .0001 | .2043 | .8696 | .4566 | .7217 |
| Group effect       | .1302 | .1410 | .0001 | .0001 | .0001 |

ISO indicates isoproterenol; BW, body weight; KW, kidney weight; LV, left ventricular; LV Path score, LV pathology score (see text for description); and LVEDP, LV end-diastolic pressure. Values are mean±SEM.

1.9±0.2, 2.8±0.2, and 3.8±0.2 (P<.001 for each group versus the control group) for 0-, 85-, 170-, and 340-mg/kg ISO groups, respectively. Because histological resolution of the damage to the myocardium occurred by 2 weeks, there was no evidence for any further increase in this pathology score with respect to time (2 to 16 weeks) within each dosage group (Table 1).

There were no differences in LV mass among ISO groups by 2 weeks after injection, suggesting that the myocardium lost to necrosis had been fully replaced (Fig 1). By 6 weeks, there was a tendency for LV mass to increase both by dose and time, a trend that became significant by 16 weeks for all ISO groups with respect to time and for the 170- and 340-mg/kg groups with respect to the control group. Because the physiological process of body growth was concurrent with the increase in LV mass, we indexed this variable, as well as others when appropriate, to body weight. An analysis of LV mass index with respect to both time and dose demonstrated that no changes occurred over time (from 2 to 16 weeks) but that within these time periods, this index increased in a dose-dependent manner: 1.85±0.02 (control group), 1.98±0.03, 2.03±0.03, and 2.12±0.03 g/kg (P<.001 for each ISO group versus the control group) for 0-, 85-, 170-, and 340-mg/kg ISO groups, respectively (Table 1). A dose-dependent increase in LVEDP was noted with a fourfold increase over the control group in the 340-mg/kg ISO group (P<.001, Table 1), but there were no significant time-dependent changes in LVEDP.

**LV Pressure-Volume Relation**

In general, there was a progressive rightward shift of the pressure-volume (per kilogram) curves through time for all of the ISO-treated groups (Fig 2). The extent of this dilatation was related to dose, as well. Indeed, whereas the LV of the 85-mg/kg ISO group had not significantly dilated by 2 weeks after injection, that of the 340-mg/kg ISO group had already achieved volumes that were ≈50% of those at 16 weeks. These pressure-volume curves were characterized through regression analysis along different pressure ranges, and slopes representing ventricular chamber stiffness constants from these different segments were compared among groups to investigate which portion of the curve had been predominantly affected. The pressure-volume curve from 3 to 30 mm Hg distending pressure demonstrated a decrease with dose in the overall chamber stiffness constant (k330, 2.39±0.05, 2.32±0.05, 2.21±0.04 [P<.05], and 2.17±0.06 [P<.01] mm Hg · kg · mL⁻¹ for 0-, 85-, 170-, and 340-mg/kg ISO groups, respectively). This change in the overall chamber stiffness constant from 3 to 30 mm Hg
Table 2. Slope of Left Ventricular Pressure-Volume Curve for Distension Pressure From 0 to 3 mm Hg

<table>
<thead>
<tr>
<th>ISO Dose Group</th>
<th>Slope of LV Pressure-Volume Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 Weeks</td>
</tr>
<tr>
<td>0 mg/kg</td>
<td>11.5±0.6 (10)</td>
</tr>
<tr>
<td>85 mg/kg</td>
<td>8.8±0.7* (10)</td>
</tr>
<tr>
<td>170 mg/kg</td>
<td>7.5±0.8* (10)</td>
</tr>
<tr>
<td>340 mg/kg</td>
<td>5.5±0.4* (10)</td>
</tr>
</tbody>
</table>

ISO indicates isoproterenol; LV, left ventricular. Values are mean±SEM. The number appearing in the parentheses after each value represents the sample size.

*P<.01 for ISO dose group vs control group (0-mg/kg group) within study period; †P<.05 vs value at 2 weeks within ISO dose group.

was primarily due to the dose-dependent decrease in the slope of the logarithmic pressure-volume segment from 3 to 10 mm Hg (k3,10, 2.52±0.06, 2.38±0.07, 2.26±0.07 [P<.01], and 2.10±0.06 [P<.01] mm Hg·kg·mL−1 for 0-, 85-, 170-, and 340-mg/kg ISO groups, respectively), whereas the slopes from 10 to 20 mm Hg (k10,20, overall mean, 2.25±0.04) and from 20 to 30 mm Hg (k20,30, overall mean, 2.55±0.05) did not change with respect to either dose or time. However, the linear portion of the curve from 0 to 3 mm Hg filling pressure (k0,3), Table 2 was marked extended, with decreases in stiffness constants with respect to both time (P<.001) and dose (P<.001). Time-dependent changes in this slope occurred for both the 170-mg/kg and the 340-mg/kg ISO groups (P<.01), although to different extents. The decreased slope of the 170-mg/kg ISO group at 6 weeks (5.5±0.3) was already present in the 340-mg/kg ISO group at 2 weeks (5.5±0.4). These results suggest that the majority of LV enlargement occurred in the early, passive filling portion of the pressure-volume curve and thus was due to structural alterations of the ventricular wall.

LV Volumes

To investigate ventricular dilatation independent of changes caused by ventricular distension (154 cases, at least 10 rats per group), ventricular volumes (Fig 3, indexed for body weight) were compared at common distension pressures. The LV volume indexed for body weight at 5 mm Hg distension pressure was selected as representative of the passive filling portion of the ventricular pressure-volume curve (ie, that part of filling associated with distension of the wall from the collapsed state with minimal engagement of the myocardium).
Although stable over time for control animals, these volumes increased in ISO-treated rats with a significant two-way interaction of time and dose \((P=.013, \text{Fig } 3\text{A})\). The intermediate- and high-dose groups demonstrated progressive time-dependent ventricular dilatation, with respective increases at 16 weeks after injection of 33% and 42% beyond the already abnormal values at 2 weeks. Indeed, when compared with the average (over time) ventricular volume at 5 mm Hg distension pressure for the control group of 0.62 mL/kg, that for the 340-mg/kg ISO group at 16 weeks was 1.36 mL/kg, a greater than twofold increase. The extent of ventricular dilatation was clearly not only time but also dose dependent, with significant increases in the average ventricular volume at 5 mm Hg distension pressure in all dose groups compared with the control group \((0.62\pm0.02, 0.78\pm0.02, 0.94\pm0.04, \text{ and } 1.21\pm0.05 \text{ mL/kg for } 0-, 85-, 170-, \text{ and } 340\text{-mg/kg ISO groups, respectively}; P<.0001\).

A similar analysis of ventricular volumes was performed at 20 mm Hg, a pressure at which the mechanical characteristics of the myocardium are more fully engaged. These volumes also increased in a time- and dose-dependent manner, with a significant two-way interaction \((P=.046, \text{Fig } 3\text{B})\). At 2 weeks after injection, there was already a significant dose effect: the mean ventricular volume at 20 mm Hg distension pressure of the highest dose group was 39% greater than that of the control group \((1.65 \text{ versus } 1.19 \text{ mL/kg, respectively})\). The time-dependent increases in ventricular volume \((20 \text{ mm Hg})\) were also remarkable; even though the 85-mg/kg ISO group was relatively stable from 2 to 16 weeks, the 170- and 340-mg/kg ISO groups continued to increase to \(>20\%\) beyond the abnormal values already present at 2 weeks. This progressive dilatation yielded volumes in the highest dose group that were 67% greater than those in the control group by 16 weeks after injection.

For each animal, an index of in vivo LV operating volume was derived from that LV volume taken from the pressure-volume curve that corresponded to the measured resting LVEDP. These operating volumes are a measure of the combined effects of ventricular distension consequent to an increase in LVEDP and ventricular structural dilatation through time. The LV operating volumes increased dramatically with dose \((\text{Fig } 3\text{C})\): the 85-mg/kg ISO group had a 73% increase in operating volume over the control group \((P<.001)\) at 2 weeks after injection, whereas the 170- and 340-mg/kg ISO groups had remarkable 166% and 279% increases, respectively \((P<.001)\). In the intermediate- and high-ISo groups, significant progressive increases in operating volume occurred from 2 to 16 weeks after injection despite the lack of change in filling pressure \((P<.001)\).

LV Mass-to-Volume Ratio and Global Diastolic Wall Stress

The ratio of LV mass to volume at a common distension pressure \((5 \text{ and } 20 \text{ mm Hg})\) decreased in both a time- and dose-dependent fashion. This ratio at a low \((5 \text{ mm Hg})\) distension pressure \((\text{Fig } 4\text{A})\) did not change over time in the control groups but decreased in a dose-dependent fashion among all ISO groups. Although there were no time-dependent changes observed in the 85-mg/kg ISO group, the 170- and 340-mg/kg ISO group demonstrated time-dependent decreases in this ratio of 40% and 27%, respectively, from 2 to 16 weeks after injection. Changes of similar magnitude occurred in the LV mass-to-volume ratios at 20 mm Hg for the 170- and 340-mg/kg ISO groups \((\text{Fig } 4\text{B})\). The ratio of LV mass to operating volume at the resting LVEDP \((\text{Fig } 4\text{C})\) demonstrated a clear dose-dependent effect for all ISO groups, as well as time-dependent changes in the intermediate- and high-dose groups, such that the ratio of LV mass to operating volume decreased by 28% through time in the 340-mg/kg ISO group, from 1.65\(\pm\)0.11 g/mL at 2 weeks to 1.19\(\pm\)0.06 g/mL at 16 weeks.

Global diastolic wall stress represents the combined effect of LV hypertrophy, chamber dilatation, and elevated filling pressures in response to the ISO-induced damage. There was a significant exponential relation between LV wall stress and the extent of damage, as assessed by the LV pathology score \((\text{Fig } 5\text{A})\), with an overall exponential regression equation of \(\log(\text{LV wall stress})=0.03+0.255 \cdot \text{LV pathology score} \left( R^2=.78, P<.0001 \right)\). However, correlation between the LV pathology score and wall stress progressively changed through time, such that the same pathology score was correlated with a higher wall stress at week 16 than at week 2 \((\text{ANCOVA}, P<.01)\). LV global end-diastolic wall stress increased in a dose-dependent manner \((\text{Fig } 5\text{B})\) by over twofold in the 85-mg/kg ISO group and by sixfold in the intermediate-dose group \((P<.001)\). The most dramatic change occurred in the 340-mg/kg ISO group, in which wall stress at 16 weeks was 15-fold greater than that in the control group. In both the intermediate- and high-ISo groups, end-diastolic wall stress progressively increased as volume increased out of proportion to mass through time, becoming 26% \((170\text{-mg/kg ISO group}, \text{Fig } 4\text{D})\).
Fig 5. Scatterplot (A) and bar graph (B) showing that left ventricular (LV) global end-diastolic wall stress (dyne/cm²×10⁶) and LV pathology score (see "Materials and Methods") in isoproterenol (ISO)-treated rats were exponentially related (n=134, A). Exponential linear regression lines for the separate time groups demonstrate a significant (P<.01) increase in LV wall stress for the same LV pathology score at week 16 compared with week 2, demonstrating a progressive change in the relation through time. LV wall stress, expressed as mean±SEM, changed significantly with respect to both time and dose (B). The bars are grouped by dose; open, shaded, and solid bars represent 2-, 6-, and 16-week groups, respectively. *P<.01 between groups; †P<.05 between groups. Note that all 170- and 340-mg/kg ISO dose groups are significantly different from their respective control group (0 mg/kg ISO) within their study period (P<.01).

P=.045) and 33% (340-mg/kg ISO group, P<.001) greater at 16 weeks when compared with the already elevated values at 2 weeks after injection.

Mortality

All rats were carefully monitored throughout the duration of study so that assessments of mortality over time within the dosage groups could be made. There were no deaths in the control (0-mg/kg ISO) group. The immediate (24-hour) mortality rate was high for all active doses: 55% in the 85-mg/kg ISO group, 66% in the 170-mg/kg ISO group, and 75% in the 340-mg/kg ISO group. Of the rats surviving to 6 weeks, the interval mortality to 16 weeks was 0% for the control group and 85-mg/kg ISO group but was 16% for the 170-mg/kg ISO group and 32% for the 340-mg/kg ISO group (Fig 6). The highest dosage group lost a significantly greater percentage of animals after the 6-week study period than did the other groups (P<.001). These immediate and interval deaths yielded overall mortality rates of 55% in the 85-mg/kg ISO group, 70% in the 170-mg/kg ISO group, and 82% in the 340-mg/kg ISO group.

Discussion

The present study has demonstrated time- and dose-dependent ventricular remodeling in response to remote graded myocardial necrosis in ISO-treated rats despite the presence of a patent coronary circulation and the absence of further myocardial insult. At 2 weeks after injection, dose-dependent myocardial necrosis was associated with commensurate dose-dependent increases in LV filling pressures, myocardial hypertrophy, and ventricular dilation. Subsequent analysis at 6 and 16 weeks after injection showed progressive ventricular enlargement and alterations of the pressure-volume relation in the face of no further pathological damage and an intact coronary vasculature. This progressive ventricular enlargement was associated with increased mortality in the intermediate- and high-dose rats, even when the assessment was started 6 weeks after the injection of ISO.

The rat model of ISO-induced myocardial necrosis has been extensively reviewed, although the exact mechanism of action remains unknown. The pathological changes resemble those of myocardial infarction with replacement fibrosis in a generally patchy, subendocardial distribution. Previous studies have shown that this effect is dose dependent and results in a deterioration of hemodynamic variables both acutely and chronically. ISO is rapidly metabolized by the monoamine oxidase and catechol-O-methyltransferase systems, and consequently, this investigation began observations at 2 weeks after the injection of ISO, avoiding any confounding acute effects and ensuring that any subsequent changes reflected the late myocardial response to the initial remote insult.

In the present study, the administration of subcutaneous ISO to rats caused dose-dependent, graded, diffuse myocardial necrosis with resultant dose-dependent increases in LV filling pressure and mass, as well as ventricular chamber enlargement evident at 2 weeks after injection. The LV pathology score increased in discrete increments, with each dose producing damage that was statistically distinct from the others. A pronounced (280%) increase in the operating volume of the high-dose group was noted at 2 weeks, but the 16% increase in LV mass was insufficient to offset this degree
of enlargement. Consequently, marked decreases in the LV mass-to-volume ratio and increases in the global diastolic wall stress were already present 2 weeks after ISO injection.

Progressive ventricular remodeling occurred well after the initial insult in the present study, but to minimize any possible effects of acute dilatation, all time-dependent variables were compared with the 2-week study groups. There was pronounced LV enlargement with time-dependent increases in ventricular volumes at common distension pressures, and the pressure-volume relation showed progression of the dose-dependent changes through time, with topographical increases in the capacitance portion of the curves but preservation of the stiffness constants at higher pressures. Progressive dose-dependent decreases in the ventricular stiffness constant over the 0 to 3 mm Hg range occurred, findings consistent with changes in ventricular structure (dilation) independent of ventricular distension. Although LVEDP did not increase beyond the 2-week value, operating volume increased dramatically. The absolute increase in LV mass at later times after injection was far less than that in volume, and consequently, the ratio of LV mass-to-volume continued to decline while global end-diastolic wall stress increased through time. Although it cannot be excluded that the marked increases in wall stress over time are produced by alterations in the strain, the stability of the filling pressure (LVEDP) underscores that the progressive increase in wall stress could not be the consequence of acute distension but rather reflects global LV enlargement. These time-dependent differences could not have been due to an increase in the severity of histological damage, because there were no significant differences in pathology scores with respect to time at 2 weeks and beyond within the dosage groups. However, the global wall stress correlated strongly with the extent of cardiac necrosis, as measured by the pathology score.

The acute mortality from the administration of ISO in the present study compared reasonably well with previously reported rates of 10%, 50%, and 90%, respectively, for the 85-, 170-, and 340-mg/kg ISO doses. However, the unstable progressive cycle of ventricular remodeling reported in the present study resulted in an increase in late mortality as well. Since no rats were removed for terminal study between 6 and 16 weeks, an analysis of mortality in rats with chronic congestive heart failure uncomplicated by ongoing ischemia was possible. The animals given higher ISO doses had a significantly greater long-term mortality, which directly correlated with the increased ventricular enlargement, demonstrating that a one-time loss of myocardium is sufficient for a worse prognosis. Thus, in the present study, the myocardial response to graded diffuse injury consisted of an unstable cycle in which progressive ventricular enlargement resulted in an increase in global wall stress and a decrease in the mass-to-volume ratio, which caused further ventricular chamber enlargement and increased late mortality.

These findings are similar to those in the rat model of myocardial infarction, wherein discrete segmental myocardial necrosis is produced by permanent occlusion of the coronary artery. LVEDPs increase rapidly in the course of myocardial infarction, and the increase in the high-dose ISO group was roughly similar to that noted previously with moderate-sized myocardial infarctions. Myocyte hypertrophy occurs early (by 3 days) after myocardial infarction, as shown by Anversa and coworkers, who found that surviving LV myocytes hypertrophied by 28% with increases in both cell length and diameter. As has been found in the rat with coronary artery ligation, ventricular chamber enlargement was directly correlated to myocardial damage in the present study. In humans, many studies have also demonstrated a relation between the extent of myocardial infarction, usually determined by the extent of akinesis and/or dyskinesis, and the degree of ventricular dilatation.

Despite the differing etiologies of the myocardial damage (diffuse versus segmental damage), the compliance characteristics of the LV chamber in the ISO-treated rats were also similar to those found in rats with discrete segmental infarctions: a rightward shift in the early capacitance portion (0 to 3 mm Hg) of the pressure-volume curve and a decrease in the overall chamber stiffness constant (3 to 30 mm Hg).

Ventricular enlargement during the first 2 weeks after ISO injection was probably the result of two distinct processes: scar formation and infarct expansion. Infarct expansion is the process of disproportionate thinning and dilatation of the acutely infarcted myocardium that begins within the first 24 hours after infarction.

The thinning of the infarcted segment is due to a combination of a decrease in the number of cells across the wall, myocyte slippage, and, to a lesser extent, cell stretch and a decrease in the intercellular space. These structural alterations lead to an increase in the basic capacity (or nondistended volume) of the heart. This pathological process has been demonstrated to occur in the rat model of coronary artery ligation and is suggested in the present study of the ISO-injected rat, as evidenced by the marked increase in LV volume at low distension pressures. Ventricular remodeling also takes place in the noninfarcted myocardium, where myocyte bundle rearrangement is the primary cause of ventricular dilatation and wall thinning and has been demonstrated on a physiological level and cellular level in the infarct model. In the present study of diffuse myocardial necrosis, changes occurred in both the necrotic segments and the residual myocardium, as evidenced by the increased ventricular volumes at high (20 mm Hg) distension pressure as well as the moderate, though insufficient, myocyte hypertrophy. The increases in LV operating volume initially may be beneficial, enabling maintenance of stroke volume with a decrease in the required extent of circumferential fiber shortening.

Although a preservation of cardiac output has been noted in rats with segmental infarcts 3 weeks after ligation (except for those with infarcts in excess of 46%), the present study, cardiac output was not preserved in the intermediate- and high-dose groups 2 weeks after ISO injection (authors' unpublished data), despite marked ventricular remodeling. However, the ventricular dilatation observed in the present study occurred predominantly in the capacitance portion of the pressure-volume curves, where such increases may not afford a mechanical advantage. In a dog model of chronic heart failure induced by multiple sequential coronary microembolizations, changes in LV shape preceded the development of profound ventricular dysfunction and dilatation, suggesting that this remodeling.
is not a manifestation of, but rather may have a causal role in, the development of ventricular dysfunction.

Thus, in our study, the ventricular remodeling that occurred 2 weeks after the production of myocardial necrosis by ISO was similar to that caused by coronary artery ligation, despite the presence of a patent coronary circulation.

Progressive time-dependent ventricular remodeling has been reported in the rat model of coronary artery ligation with a discrete segmental loss of myocardium and is reported as well in the present study for ISO-treated rats with graded diffuse myocardial fibrosis. The LV volume indexed for body weight at 20 mm Hg distension pressure for moderate-sized infarcts (30% to 45%) was 0.5 mL/kg at 106 days, which is similar to the value of 2.1 mL/kg found in the present study at 16 weeks in the 340-mg/kg ISO group. This ventricular enlargement in the moderate-sized infarct group was not matched by the increases in heart weight, resulting in a progressive decrease in the ratio of mass to volume through time, with a ratio at 20 mm Hg distension pressure of 1 g/mL at 106 days. In the present study, the ratio for the 340-mg/kg ISO group was 1.07 g/mL at a similar time after the acute insult (16 weeks, or 112 days). Although there were no changes in the chamber stiffness constants from 2.5 to 10 and from 15 to 30 mm Hg on the pressure-volume curve at 26 days in rats with infarcts up to 43±1% compared with control rats (except for a decrease in large-sized infarcts [50%]), a significant decrease in the overall chamber stiffness constant (from 2.5 to 30 mm Hg) was found in rats with moderate to large infarcts by 106 days, as also observed in the present study. The mechanism of this progressive ventricular enlargement in our study is suggested by the lack of sufficient compensatory myocardial hypertrophy, supporting the hypothesis that it is primarily due to side-to-side slippage of myocardial cellular elements and secondarily due to modest myocyte cell lengthening, or a combination of both. Ventricular enlargement has been noted to portend a poor prognosis in animals and in humans, in whom an incremental ventricular enlargement of as little as 25 mL in end systole increased the relative risk of death in an exponential manner. Other studies confirm these findings that although ventricular dilatation may initially serve to increase cardiac output, it may have long-term detrimental effects—an increase in myocardial mass, wall stress, and myocardial oxygen demand, leading ultimately to an increase in mortality as its penalty.

In conclusion, the present study demonstrates that remote myocardial damage can lead to progressive ventricular remodeling despite the presence of a patent coronary circulation and absence of recurrent insult. The extent of ventricular remodeling was directly related to the extent of remote myocardial injury, providing further support for the importance of infarct limitation in the prevention of subsequent development of heart failure. However, our findings do not reduce the important role that ongoing ischemia or myocardial damage can play in the process of ventricular remodeling, as has been demonstrated in recent studies. The acute damage caused by ISO resulted in marked ventricular enlargement, a decrease in the ratio of LV mass to volume, and an increase in wall stress that progressed through time. An increase in long-term mortality was also seen in rats from the intermediate- and high-isoproterenol dose groups, a finding that correlated with the unstable positive-feedback cycle of ventricular enlargement and increasing wall stress observed in these groups. These changes in response to an initial diffuse loss of myocytes were similar to those reported in animal models and patients with segmental myocardial damage and may represent a common adaptive mechanism of the ventricle to the myocardial damage.

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