Effect of Prolonged Nitrate Therapy on Left Ventricular Remodeling After Canine Acute Myocardial Infarction

Bodh I. Jugdutt, MBChB, MSc; Mohammad I. Khan, MBBS

Background Prolonged nitrate therapy during healing between 2 days and 6 weeks after anterior myocardial infarction has the potential for limiting further left ventricular remodeling (or changes in topography) and preserving function. Long-term therapy throughout healing over 6 weeks might be more beneficial than short-term therapy over the first 2 weeks after infarction.

Methods and Results The effect of prolonged nitrate therapy between 2 days and 6 weeks during healing after infarction on serial parameters of ventricular remodeling (scar expansion, scar thinning, ventricular dilation, and hypertrophy) and function (asynchrony or akinetic plus dyskinesia and ejection fraction) by serial two-dimensional echocardiography, hemodynamics, postmortem topography (computerized planimetry, geometric maps, and radiographs), and collagen content (hydroxyproline) was studied in 64 instrumented dogs randomized 2 days after left anterior descending coronary artery ligation to various nitrate regimens (n=32) over the first 2 weeks (subgroup 1: 2% transdermal nitroglycerin at 8 AM and 4 PM, n=6; subgroup 2: 2% transdermal nitroglycerin plus 2.6 mg of sustained-release oral nitroglycerin at 8 AM, 3 PM, and 10 PM, n=5; subgroup 3: oral isosorbide dinitrate, 30 mg at 8 AM and 4 PM, n=11) or 6 weeks (subgroup 4: isosorbide dinitrate, n=10) and in matching controls (n=32). Nitrate therapy reduced left atrial pressure, mean arterial pressure, and the rate-pressure product compared with controls over the 6 weeks. Postmortem scar mass and hydroxyproline were similar in control and nitrate groups. However, scar stretching and thinning, cavity dilation, noninfarct wall hypertrophy, and apical bulging were less with nitrates, especially in the long-term subgroup 4. In vivo remodeling parameters between 2 days and 6 weeks after ligation showed that, compared with controls, nitrate therapy prevented further stretching of the asynergic segment, decreased the expansion index, decreased further scar thinning, prevented the increase in ventricular volumes, reduced the frequency of ventricular aneurysm, prevented the increase in ventricular mass, reduced the extent of asynergy, and improved ejection fraction. Although the beneficial effect on topography and function was seen in all nitrate subgroups, the overall benefit was greater with long-term therapy over 6 weeks (subgroup 4) than short-term therapy confined to the first 2 weeks (subgroups 1, 2, and 3).

Conclusions Prolonged nitrate therapy, in various regimens during healing after infarction, effectively reduced left ventricular loading and prevented infarct thinning, further infarct expansion, progressive ventricular dilation, and the increase in mass. These effects were associated with decreased asynergy and improved ejection fraction. The beneficial effects were greater with long-term therapy over 6 weeks than short-term therapy over the first 2 weeks. (Circulation. 1994;89:2297-2307.)

Key Words • infarction • hypertrophy • collagen

Imitation of postinfarction left ventricular remodeling, or the alterations in topography that often impact negatively on function and survival, is becoming recognized as an important therapeutic goal.1-4 Nitrates after infarction have the potential to salvage left ventricular myocardium, geometry, and function because of their ventricular unloading and flow-promoting properties.5 Despite century-old warnings about nitrate-induced hypotension in acute myocardial infarction6-8 and the development of tolerance with chronic use,9,10 nitrates were successfully applied clinically to treat left ventricular failure11-13 or to prevent its development14,15 in acute myocardial infarction. Short-term, low-dose intravenous nitroglycerin infusion early during acute infarction was shown to improve left ventricular function and myocardial perfusion in dogs16 and humans.17 In one clinical study, early infusion produced prompt and marked decrease in left ventricular asynergy, and the beneficial effect persisted at 10 days.18 Short-term intravenous nitroglycerin infusion therapy during acute infarction in the dog19 was later shown to reduce infarct thinning, a very early manifestation of ventricular remodeling with stretching, thinning, and bulging of the infarct zone.1-4 In the clinical setting, intravenous nitroglycerin infusion over the first 48 hours after infarction was also found to limit infarct expansion and improve left ventricular function and outcome.20 Preliminary clinical reports21-23 have suggested that prolonged left ventricular unloading during infarction and healing, with reduction of preload, afterload, chamber size, and wall stress by chronic nitrate therapy, might limit both early infarct expansion and further progressive remodeling, but this has not been studied systematically in a well-defined dog model. Nitroglycerin therapy between 2 and 7 days after infarction in the dog reduced expansion and thinning and improved function, mechanical strength, and resistance to distension at 6 weeks. Intermittent and eccentric

Received October 25, 1993; revision accepted January 14, 1994.

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**TABLE 1. Nitrate Therapy and Control Subgroups**

<table>
<thead>
<tr>
<th>Control Subgroup (n)</th>
<th>Nitrate Subgroup (n)</th>
<th>Nitrate Strategy</th>
<th>Nitrate Preparation</th>
<th>Nitrate Dosing Schedule</th>
<th>Duration of Nitrate Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (6)</td>
<td>1 (6)</td>
<td>Early, intermittent</td>
<td>TDN (2%)</td>
<td>8 AM, 4 PM</td>
<td>First 2 wk</td>
</tr>
<tr>
<td>2 (5)</td>
<td>2 (5)</td>
<td>Early, sustained</td>
<td>TDN (2%) and NTG SR (2.6 mg)</td>
<td>8 AM, 3 PM, and 10 PM</td>
<td>First 2 wk</td>
</tr>
<tr>
<td>3 (11)</td>
<td>3 (11)</td>
<td>Early, intermittent</td>
<td>ISDN (30 mg)</td>
<td>8 AM, 4 PM</td>
<td>First 2 wk</td>
</tr>
<tr>
<td>4 (10)</td>
<td>4 (10)</td>
<td>Early, intermittent, and prolonged</td>
<td>ISDN (30 mg)</td>
<td>8 AM, 4 PM</td>
<td>6 wk</td>
</tr>
</tbody>
</table>

TDN indicates transdermal nitroglycerin ointment; NTG SR, nitroglycerin sustained-release tablet; and ISDN, isosorbide dinitrate tablet.

dosage schedules have been suggested for reducing tolerance in angina patients, but the effect of such schedules on postinfarct remodeling has not been systematically studied. Whether short-term therapy before the collagen plateau or long-term therapy throughout postinfarct healing might be equally effective has not been determined.

The primary aim of this study was to test the hypothesis that left ventricular unloading with four regimens of prolonged nitrate therapy during the healing process between 2 days and 6 weeks after completion of anterior acute myocardial infarction might limit left ventricular remodeling and improve function assessed by quantitative two-dimensional echocardiography. A secondary aim was to test the effect of pulses of nitrate therapy for the first 2 weeks and 6 weeks after infarction.

**Methods**

**Experimental Preparation**

All experiments were approved by the institutional animal welfare committee and conformed to the guiding principles of the American Physiological Society. Seventy-five healthy mongrel dogs (weight, 16 to 29 kg) of either sex were instrumented through a left lateral thoracotomy under general anesthesia (sodium pentobarbital, 30 mg/kg IV), as described previously. Polyethylene catheters were inserted in the external jugular vein, internal carotid artery, and left atrium, filled with heparinized saline, and their ends exteriorized behind the neck. A silk ligature was placed around the mid left anterior descending coronary artery, between the first and second diagonal branches, and tied. Metal beads were sutured on the anterior, lateral, and posterior epicardial surfaces in the short-axis plane at the mid left ventricular level for consistent echocardiographic orientation for serial topography. The pericardium and chest were then closed. Penicillin (1 million units) and streptomycin (1 g) were given intramuscularly, and the dogs were returned to their cages.

**Experimental Design**

Two days after coronary artery ligation, the 70 healthy survivors were randomized to nitrate therapy (n=35) and matching control subgroups (no treatment, n=35): subgroup 1 (6 control, 6 nitrate), subgroup 2 (6 control, 6 nitrate), subgroup 3 (12 control, 12 nitrate), and subgroup 4 (11 control, 11 nitrate). The dogs were allowed free access to fluids, and no attempt was made to treat heart failure by fluid restriction or pharmacotherapy. At 6 weeks, the 64 surviving dogs (Table 1) were anesthetized, and the hearts were arrested in diastole with an overdose of intravenous potassium chloride, excised, washed in normal saline solution, and weighed. Blood samples were taken for monitoring blood gases, hemogroms, and electrolytes.

Measurements During Healing

As described previously, serial ECGs (Gould recordor), hemodynamics (Statham P23Db for left atrial and arterial pressures), and two-dimensional echocardiograms (Toshiba SSH-65A, 3.5 MHz transducer) were recorded with the dogs in the conscious state and standing in a sling for support at 2 days before therapy, weekly during therapy, again after therapy, and before the dogs were killed. In addition, baseline echocardiograms were obtained before and after surgery. Echocardiograms were stored on 0.5-in. VHS videotape for later analysis. As for human studies, the parasternal long-axis; five parasternal short-axis views from base to apex at mitral, chordal, mid papillary, low papillary, and apical levels; and the apical four- and two-chamber views.

Postmortem Measurement of Scar Size, Geometry, and Collagen

Postmortem coronary arteriography was performed on fresh hearts using simultaneous pressure-controlled injections of all coronary arteries with a mixture of barium sulfate and gelatin, as described previously. The hearts were then fixed in distension (15-cm pressure head) to preserve diastolic proportions with 10% phosphate-buffered formalin solution for 48 hours and radiographed in two perpendicular planes. Five transverse sections (1 to 1.5 cm thick) with four equally spaced sections below the level of the ligation to the apex were then made and radiographed. Boundaries of the occluded bed or anatomic risk region were marked on section radiographs by consensus of two observers at the watersheds between terminal branches of the visualized vessels. The sections were weighed after removing the right ventricle and other extraneous tissue, materials, and beads. Outlines of the rings, occluded zones, and infarct scars were made on plastic overlays. Computerized planimetry (Hewlett-Packard 9835A computer and 9874A digitizer interfaced with a VAX 750 computer) was used to derive the following parameters, as described previously: areas of the left ventricular ring and cavity, infarct scar, occluded bed, and noninfarcted myocardium; thicknesses of infarct and left ventricular ring; endocardial lengths of infarct and noninfarct segments; circumferences of the ring and infarct; mass of infarct and occluded bed by relating the average areas (top and bottom surfaces) to the weight of each ring; total masses of infarct and occluded bed for each heart by summing values for each ring; Thinning ratio (average thickness of infarcted wall to average thickness of the normal wall); expansion index (ratio of endocardial lengths of infarct to noninfract containing segments demarcated by papillary muscle landmarks); geometric maps of the infarct and risk region for each transverse section for each left ventricle; and average maps of these data for each transverse section for each group. In addition, contours of the left ventricular epicardium and endocardium were made from the whole-heart radiographs and digitized to map topography and measure the area and depth of the apical bulge in the long axis, and the digitized...
TABLE 2. Hemodynamics In Control and Nitrate Groups

<table>
<thead>
<tr>
<th>Timing</th>
<th>Heart Rate, beats per minute</th>
<th>Mean Left Atrial Pressure, mm Hg</th>
<th>Mean Arterial Pressure, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nitrate</td>
<td>Control</td>
<td>Nitrate</td>
</tr>
<tr>
<td></td>
<td>112±3</td>
<td>112±3</td>
<td>5±0</td>
</tr>
<tr>
<td></td>
<td>147±3†</td>
<td>145±4†</td>
<td>14±1†</td>
</tr>
<tr>
<td></td>
<td>131±4†</td>
<td>129±4†</td>
<td>16±1</td>
</tr>
<tr>
<td></td>
<td>116±3†</td>
<td>106±3†</td>
<td>6±1†</td>
</tr>
<tr>
<td></td>
<td>117±4</td>
<td>112±4</td>
<td>6±1*</td>
</tr>
<tr>
<td></td>
<td>110±4*</td>
<td>93±4</td>
<td>7±1*</td>
</tr>
<tr>
<td></td>
<td>108±3</td>
<td>100±4</td>
<td>7±0*</td>
</tr>
<tr>
<td></td>
<td>101±3*</td>
<td>87±5</td>
<td>8±1*</td>
</tr>
<tr>
<td></td>
<td>98±4</td>
<td>98±4</td>
<td>8±1*</td>
</tr>
<tr>
<td>Percent change (day 2 to 6 weeks)</td>
<td>-20±4*</td>
<td>-12±6</td>
<td>-50±15*</td>
</tr>
</tbody>
</table>

*P<.05, significance of difference comparing corresponding values in nitrate and control groups; †P≤.05, significance of difference compared with preceding value within the group.

Data then were reduced to average maps for each group. Histopathology was done on a 5-mm slice from the middle of the infarcted zone. Triplicate 5-μm-thick sections were stained with hematoxylin and eosin, Mallory's stain, or Masson's trichrome, respectively, and examined for infarction and collagen. As described previously,25 transmural myocardial tissue samples were taken from the center, border, and margin regions of the infarct scar and the center of the nonoccluded bed, weighed (100 to 200 mg), and processed for measurement of hydroxyproline content as a marker for collagen in milligrams per gram of dry tissue weight.

Analysis of Echocardiograms

Coded echocardiograms were analyzed on video playback in double-blind fashion by two independent observers at the end of the studies, as described previously.20 Briefly, endocardial and epicardial outlines of the left ventricular images at end diastole and end systole were traced with a light pen (Franklin Quantic 1200 review station or Disonics CardioRevue Center), corrected on-to-and-fro playback over at least three consecutive cycles, and copied on plastic overlays. Anatomic landmarks (papillary muscle, right and left ventricular junctions) were indicated on the tracings. Markings of asynergy, defined as akinesis (no systolic inward motion and thickening), dyskinesis (systolic outward motion and thinning), or both, were made on each endocardial diastolic outline by careful visual assessment of motion and thickening on repeated video playbacks. The circumferential extents on each short-axis view were then digitized (Hewlett-Packard 9878A and 9835A) and used to compute total endocardial surface area of left ventricular asynergy using three-dimensional reconstruction.20,28,30 Outlines from five short-axis and two long-axis views were used to compute volumes by means of the modified Simpson's rule.20 Global ejection fraction was calculated as (end-diastolic volume minus end-systolic volume) divided by end-diastolic volume. The interobserver error was less than 5% in marking asynergy, segment length, wall thickness, and areas of outlines, in agreement with previous studies.20,28,30 Topographic measurements were made on end-diastolic outlines of short-axis in vivo echocardiographic images at the papillary level. Expansion index was computed as the ratio of the lengths of the asynergy-containing and the nonasynergy-containing segments. Thinning ratio was computed as the ratio of the average thicknesses of the asynergic and nonasynergic zones. Regional area ejection fraction was calculated at the same level as (end-diastolic area minus end-systolic area) divided by end-diastolic area. The degree of regional bulging in the asynergic zone was characterized at the short-axis papillary level by its area \( A_d \), depth \( r_d \), and the peak distortion index \( P_d \), as described previously.26,30 Left ventricular aneurysm was defined as the presence of diastolic bulge with further bulging and thinning in systole on the short-axis views at the papillary and apical levels and/or the apical four- and two-chamber views. Left ventricular mass was calculated from the volume of myocardium (difference in volumes of epicardial and endocardial shells at end diastole) multiplied by an assumed specific gravity of 1.05 g/mL.

Statistics

Data at different steps were coded and analyzed in blinded fashion at the end. The following statistical tests were used: ANOVA for the significance of difference within and between groups or subgroups; linear regression analysis by the least square fit method and the significance of r values and slopes by ANOVA; 2×2 χ² and Fisher's exact tests for the significance of difference in event frequency between groups; and repeated-measures ANOVA for comparing serial data within groups. Results are presented as mean±SE. Statistical significance was set at P<.05.

Results

Data from 64 dogs that survived to the day of scheduled killing and that were randomized to control (n=32) and nitrate (n=32, Table 1) groups are presented. Five dogs that died within 2 days of coronary ligation and another 6 dogs that died between day 3 and the scheduled killing were excluded. Data for the control subgroups were similar (P=NS), so pooled control data are presented. For nitrate, the data are either pooled for all subgroups or presented for the pooled short-term (over 2 weeks) therapy subgroups 1, 2, and 3 weeks and long-term (over 6 weeks) therapy subgroup 4 when differences were significant.

Effect on Hemodynamics

Heart rate, mean left atrial pressure, and mean arterial pressure in the two groups were similar after ligation (Table 2). There were no marked differences in heart rate between the two groups over the 6 weeks. Nitrate therapy resulted in a prompt and persistent
TABLE 3. Infarct Scar Size in Nitrate and Control Groups

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Infarct Scar Mass, g</th>
<th>Risk Region Mass, g</th>
<th>LV Mass, g</th>
<th>Infarct Scar/Risk, %</th>
<th>Risk Region/LV Mass, %</th>
<th>Infarct Scar/LV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate (32)</td>
<td>7.3±1.1</td>
<td>17.4±1.6</td>
<td>81.5±2.7</td>
<td>36.7±3.8</td>
<td>21.3±3.8</td>
<td>9.1±1.2</td>
</tr>
<tr>
<td>Control (32)</td>
<td>7.5±0.8</td>
<td>18.0±1.4</td>
<td>77.2±3.0</td>
<td>43.0±3.2</td>
<td>23.9±1.8</td>
<td>10.2±1.1</td>
</tr>
</tbody>
</table>

LV indicates left ventricular. P=NS for all statistical comparisons between groups.

decrease in left atrial pressure to levels lower than in the control group. Nitrate therapy also resulted in a significant decrease in mean arterial pressure between day 2 and 6 weeks (115±2 versus 106±2 mm Hg, P<.001), and the level was lower than in controls at 6 weeks (106±2 versus 114±2 mm Hg, P<.05). Left atrial pressure and mean arterial pressure increased slightly (P<.05) from the preceding values after stopping therapy in all nitrate subgroups but remained lower than in the control subgroups, indicating that tolerance was only partial with the different regimens. Thus, left atrial pressure rose from 6.8±1.1 to 7.8±0.9 mm Hg (P<.05) in nitrate subgroup 2 (sustained therapy) but did not change in control subgroup 2 (10 versus 10 mm Hg). Corresponding mean arterial pressure increased from 104±7 to 116±2 mm Hg (P<.001) in nitrate subgroup 2 but decreased in the control subgroup 2 (121±6 versus 116±4 mm Hg, P<.05). The rate-pressure product (heart rate times mean blood pressure in beats per minute times mm Hg) decreased during nitrate therapy, the percent change over 6 weeks being greater for the combined nitrate group than for controls (−24±4% versus −7±6%, P<.025) and greater for the long-term than short-term subgroups (−36±8% versus −19±4%, P<.05).

Effect on Scar Size and Collagen

There was no difference in the mass of the infarct scar or risk region at 6 weeks (P=NS) between nitrate and control groups (Table 3) in grams relative to left ventricular mass or the mass of the risk region. There was also no difference in scar mass or risk region size among the subgroups. Also at 6 weeks, infarct transmurality was similar in the two groups and averaged 62%. Myocardial hydroxyproline content in infarct and normal zones and the hydroxyproline gradient across the infarct scars were similar in nitrate and control groups (Table 4). Histology revealed similar scar tissue in both groups. Long- and short-term nitrate subgroups did not differ in infarct mass, transmurality, histopathology, or hydroxyproline content in noninfarct, infarct border, or infarct margin zones, but infarct-center hydroxyproline concentration was slightly higher with long-term therapy (49 versus 32 mg/g, P<.1).

Effect on Scar Topography

Computer-generated average geometric maps from the five left ventricular rings from base to apex for combined control and nitrate groups indicated the following differences: (1) less infarct expansion with smaller angular extents of the scar, (2) less infarct thinning with greater thickness of the midinfarct wall, (3) less reactive hypertrophy with less thickness of the noninfarct wall, and (4) less dilation with smaller cavity area. However, these differences were more marked with long-term than short-term nitrate therapy (Fig 1). Thus, main topographic measurements from the mid left ventricular region (ring 3) for the controls and long-term nitrate subgroups were infarct wall thickness, 11.5 versus 15.1 mm, P<.01; angular extent of scar, 130° versus 90°, P<.001; cavity area, 3.8 versus 1.8 cm², P<.001; noninfarct wall thickness, 17 versus 14 mm, P<.05.

Effect on Postmortem Apical Bulging

Apical bulging in the long axis (Fig 2) on contours from postmortem radiographs of hearts arrested in diastole was greater in the control group than in nitrate subgroups. Detailed measurements from average maps for the combined control and nitrate groups were depth r₁, 6.2±0.5 versus 2.1±0.5 mm, P<.001; area A₁, 2.15 versus 1.26 cm², P<.001; peak shape distortion index P₁, 6.8 versus 1.9 mm, P<.001; endocardial segment length l₁, 2.4 versus 1.5 cm, P<.001; infarct wall thickness, 5.7 versus 7.0 mm, P<.05; cavity area, 14.2 versus 12.7 cm², P<.1. In Fig 2, the apical bulge on the average endocardial contours was smaller for the long-term than short-term nitrate subgroups. Thus, the area Aₐ of the bulge for the corresponding nitrate subgroups in Fig 2 were 0.7 versus 1.8 cm² (P<.01).

Effect on In Vivo Changes in Infarct Expansion and Thinning During Healing

The control group and the nitrate subgroups showed similar degrees of infarct expansion (Fig 3) on the postligation baseline echocardiograms at 2 days. Between preligation and 2 days after ligation, the expansion index increased (P<.01) from 1.9 to 2.2 in the nitrate group and 1.7 to 2.0 in the control group. During postinfarct healing between 2 days and 6 weeks, expansion index increased by a further 3% (P<.025) in the control group and decreased by 6% (P<.025) in the nitrate group, but the persistent decrease was mainly due to the continued decrease in the long-term nitrate subgroup between 3 and 6 weeks (Fig 3). The infarct-containing segment length decreased by 3% in the nitrate group but increased by 9% in the control group.

TABLE 4. Regional Hydroxyproline Content

<table>
<thead>
<tr>
<th>Region</th>
<th>Nitraté (n=32)</th>
<th>Control (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal zone</td>
<td>5.2±0.2</td>
<td>5.0±0.3</td>
</tr>
<tr>
<td>Infarct scar border</td>
<td>8.8±0.5</td>
<td>9.7±0.9</td>
</tr>
<tr>
<td>Infarct scar margin</td>
<td>19.1±2.1</td>
<td>28.7±2.9</td>
</tr>
<tr>
<td>Infarct scar center</td>
<td>39.2±3.4*</td>
<td>40.8±3.6*</td>
</tr>
</tbody>
</table>

*P<0.05 compared with values in other regions within the group. Values are in milligrams per gram of dry weight.
Overall, the change in noninfarct-containing segment length did not differ significantly between the two groups. However, the upward trend in lengthening of the infarct- and noninfarct-containing segments between 3 and 6 weeks in the combined nitrate group was due to increases in the short-term nitrate subgroups 1, 2, and 3 (Fig 3).

Effect on In Vivo Changes in Thinning During Healing

The control group and nitrate subgroups showed similar degrees of infarct thinning (Fig 4) on the baseline echocardiograms at 2 days after ligation. Between pretreatment and 2 days after ligation, the thinning ratio decreased ($P<.001$) from 1.00 to 0.71 in the nitrate group (before therapy) and 0.99 to 0.71 in the control group. During healing, between 2 days and 6 weeks, the thinning ratio decreased by a further 28% ($P<.001$) in the control group but increased by 20% ($P<.001$) in the combined nitrate group, with no difference between the short-term and long-term subgroups (Fig 4). Infarct wall thickness decreased further by 24% ($P<.001$) in controls but increased by 11% ($P<.05$) with nitrates. The noninfarct wall thickness showed an overall increase by 7% ($P<.05$) in controls but did not change with nitrates. No significant thinning was detected in the nitrate subgroups 1, 2, and 3 between 2 and 6 weeks. However, an additional 8% decrease in infarct wall thickness (from $-15.5\%$ to $-23.6\%$) occurred during that interval in the control group (Fig 4) and subgroups. The percent changes in the infarct segment length, expansion index, infarct wall thickness, and thinning ratio during healing were markedly different between nitrate and control groups (Figs 3 and 4) as well as in the corresponding subgroups (Table 5).

Effect on Changes in Left Ventricular Volumes and Ejection Fraction In Vivo

Left ventricular end-diastolic (80 versus 89 mL) and end-systolic volumes (45 versus 53 mL) in the control and nitrate groups were similar ($P=NS$) at the postligation baseline (Fig 5). During healing, the volumes increased in the controls but decreased in the nitrate group ($P<.001$). Global left ventricular ejection fraction was equally depressed in the two groups at baseline (41% versus 43%) and improved to a greater extent with nitrates than controls, the percent changes by 6 weeks being 29% versus 15% ($P<.01$) (Fig 5). The trend for diastolic and systolic volumes to increase in the nitrate group between 3 and 6 weeks, similar to that in the control group, was due to increases in the short-term nitrate subgroups during that interval (Fig 5).
Effect on Changes in Regional Dysfunction and Bulging In Vivo

Similar regional left ventricular dysfunction was present at the baseline postligation echocardiogram in the control and nitrate groups. Thus, total left ventricular asynergy at 2 days was 27% in the combined nitrate group and 21% in the control group. The corresponding values for the circumferential extent of asynergy were 38% and 35%, respectively. Total and circumferential asynergy did not change significantly during healing in the control group but decreased markedly (P<.001) in the nitrate subgroups (Fig 6). The corresponding decreases in the parameters for the combined nitrate group were by 53% and 60%, respectively. There was no significant blunting of the decrease in circumferential or total asynergy in short-term nitrate subgroups 1, 2, and 3 that were off therapy between 2 and 6 weeks. Changes in diastolic and systolic areas and the area ejection fraction (Fig 6) followed the trend in volumes and global ejection fraction shown in Fig 5. Indexes of regional diastolic bulging of asynergic zones in the short-axis echocardiographic images were similar for the two groups at baseline and increased in the controls but decreased with nitrates. Thus, the frequency of left ventricular apical aneurysms on the echocardiograms before the animals were killed was less in the nitrate compared with the control group (8 of 32 versus 30 of 32; \( \chi^2=28.57, P<.001 \)) and agreed with the frequency of apical diastolic bulging on postmortem radiographs. There was no significant difference in in vivo apical aneurysm frequency between short-term and long-term subgroups (3 of 22 versus 5 of 10).

Effect on Changes in Left Ventricular Mass In Vivo

Although postmortem left ventricular mass was not significantly different for the two groups (Table 3), left ventricular mass from echocardiograms before the animals were killed correlated with postmortem mass \( (y=0.86x+15.51, r=.82, \text{SEE}=9.41, P<.001) \). The mass at the baseline postligation echocardiogram was similar in control and nitrate groups (92.3±4.3 g vs 101.5±3.6 g, \( P=\text{NS} \)). However, the echocardiographic left ventricular mass in the control group increased slightly from 92.3 to 96.8 g by 1 week and decreased slightly by 6 weeks to 93.6 g. In contrast, the echocardiographic left ventricular mass in the nitrate group decreased over the first week from 101.5 to 94 g (\( P<.01 \)) by 1 week and decreased further to 90 g (\( P<.005 \)) by 6 weeks. The percent change in mass from the postligation baseline over the first week and the first 6 weeks for the two groups and the nitrate long-term and short-term subgroups are depicted in Fig 7. The decrease in mass by 6 weeks was greater (\( P<.05 \)) in the long-term than the short-term nitrate subgroups (Fig 7).

Effect of Duration of Nitrate Therapy

Overall, the improvement in remodeling and functional parameters was greater with long-term therapy over 6 weeks (subgroup 4) than with short-term therapy confined to the first 2 weeks (subgroups 1, 2, and 3). This is illustrated in the summarized data for subgroups 1, 2, and 3 combined versus subgroup 4 for nitrate and control groups in Table 5.
Discussion

The new major finding in this study is that prolonged nitrate therapy during healing between 2 days and 6 weeks after anterior acute myocardial infarction limits left ventricular remodeling and preserves left ventricular function. The second new finding is that long-term nitrate therapy over 6 weeks produced greater benefit than short-term therapy over the first 2 weeks. Thus, healing over the first 6 weeks after infarction in the canine model was associated with progressive left ventricular remodeling manifested by early infarct expansion, late thinning, aneurysm formation, left ventricular enlargement, persistent left ventricular dysfunction, and mild increase in left ventricular mass. Nitrate therapy begun 2 days after infarction resulted in limitation of further infarct expansion, limitation of late infarct thinning, less diastolic bulging, a lower frequency of aneurysm, limitation of ventricular enlargement, improvement of regional and global systolic function, and a mild decrease in left ventricular mass.

These beneficial effects of nitrate therapy were associated with a sustained decrease in preload, a sustained decrease in ventricular size, a less sustained decrease in afterload, a mild decrease in heart rate, and a decrease in the rate-pressure product (an index of myocardial work). Left atrial pressure, an index of preload, decreased by 17%. Mean arterial pressure, an index of afterload, decreased by 7%. Left ventricular enlargement was prevented. Infarct scar size and risk region size were similar in nitrate and control groups. Infarct and noninfarct collagen content were also similar in the two groups. Therapy was begun 2 days after ligation, by which time the infarction process would have been completed. The different nitrate regimens were all effective, although the effect of intermittent therapy over 6 weeks was more marked than that of the other regimens over the first 2 weeks after infarction.

Critique and Limitations of the Model

In this study, quantitative two-dimensional echocardiograms were used to follow temporal changes in left ventricular topography and dysfunction by serial interrogations over 6 weeks during postinfarction healing. Temporal changes in histopathology, which were studied previously in this model and would require killing of the dogs at intervals over 6 weeks, were not done. Precautions were taken in quantifying remodeling. For example, postmortem remodeling parameters were measured on hearts that were arrested in diastole and fixed under similar distending pressure for 48 hours. In vivo remodeling parameters were measured with all dogs in the conscious state at the same short-axis level and with the pericardium closed. To ensure survival over 6 weeks for serial in vivo measurements, a mid left...
TABLE 5. Effect of Duration of Nitrate Therapy on Remodeling and Function

<table>
<thead>
<tr>
<th></th>
<th>Nitrate Subgroups 1, 2, 3 (n=22)</th>
<th>Nitrate Subgroup 4 (n=10)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>2 d</td>
<td>6 wk</td>
</tr>
<tr>
<td>Diastolic volume, mL</td>
<td>88±3</td>
<td>98±4</td>
</tr>
<tr>
<td>Systolic volume, mL</td>
<td>53±2</td>
<td>48±3</td>
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<tr>
<td>Ejection fraction, %</td>
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<td>49±2</td>
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<tr>
<td>Infarct or scar segment length, mm</td>
<td>87±3</td>
<td>90±3</td>
</tr>
<tr>
<td>Noninfarct segment length, mm</td>
<td>45±1</td>
<td>47±1</td>
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<tr>
<td>Expansion index</td>
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<td>2.0±1</td>
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<tr>
<td>Infarct or scar wall thickness, mm</td>
<td>7.5±3</td>
<td>8.7±3</td>
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<tr>
<td>Noninfarct wall thickness, mm</td>
<td>10.3±2</td>
<td>9.7±1</td>
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<td>Thinning ratio</td>
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<td>.87±.04</td>
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<tr>
<td>Circumferential asynergy, %</td>
<td>39±2</td>
<td>15±2</td>
</tr>
<tr>
<td>Total asynergy, %</td>
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<td>15±1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Control Subgroups 1, 2, 3 (n=22)</th>
<th>Control Subgroup 4 (n=10)</th>
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<tbody>
<tr>
<td></td>
<td>2 d</td>
<td>6 wk</td>
</tr>
<tr>
<td>Diastolic volume, mL</td>
<td>83±5</td>
<td>95±5</td>
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<tr>
<td>Systolic volume, mL</td>
<td>49±4</td>
<td>50±3</td>
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<tr>
<td>Ejection fraction, %</td>
<td>41±2</td>
<td>45±2</td>
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<tr>
<td>Infarct or scar segment length, mm</td>
<td>111±3</td>
<td>123±2</td>
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<tr>
<td>Noninfarct segment length, mm</td>
<td>60±2</td>
<td>65±2</td>
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<tr>
<td>Expansion index</td>
<td>1.9±.1</td>
<td>2.1±.1</td>
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<tr>
<td>Infarct or scar wall thickness, mm</td>
<td>6.9±.3</td>
<td>5.1±.2</td>
</tr>
<tr>
<td>Noninfarct wall thickness, mm</td>
<td>10.2±.1</td>
<td>10.4±.1</td>
</tr>
<tr>
<td>Thinning ratio</td>
<td>.88±.03</td>
<td>.49±.02</td>
</tr>
<tr>
<td>Circumferential asynergy, %</td>
<td>24±2</td>
<td>22±2</td>
</tr>
<tr>
<td>Total asynergy, %</td>
<td>17±1</td>
<td>14±1</td>
</tr>
</tbody>
</table>

Absolute values at 2 days and 6 weeks and percent change between these intervals are shown.
*P<.05, significance comparing percent change in nitrate subgroups 1, 2, and 3 with subgroup 4.
†P<.025, significance of percent change compared with nitrate subgroups 1, 2, and 3.
‡P<.05, significance of percent change compared with nitrate subgroup 4.

Although serial changes in collateral blood flow were also not measured, the possibility that initial infarct size might have changed differentially in the nitrate versus control groups or subgroups between 2 days and 6 weeks seems unlikely because the final scar sizes at 6 weeks were similar for those corresponding groups and subgroups.

Mechanisms of Nitrate Benefit During Infarct Healing

The potential mechanisms for the beneficial effect of nitrates on ventricular remodeling after infarction have been reviewed and are complex. The findings in this study are consistent with the notion that the beneficial effects of nitrate were due to left ventricular unloading as a result of dilation of arterial and venous beds. The decrease in preload, afterload, cardiac work, and chamber size probably decreased wall stress (by virtue of the Laplace law) and the mechanical deformation and distension forces, thereby reducing infarct bulging and global cavity dilation. It is also possible that nitrates might have increased nutrient collateral blood flow to the healing infarct zone and the spared myocardium in the occluded bed and preserved flow in the nonoccluded bed. The
combination of decreased wall stress, increased collateral flow to border zone tissue, and preserved flow to normal myocardium also might have preserved the integrity of the collagen matrix in noninfarcted tissue, including that in the subepicardial rim. There was no adverse effect of nitrates on collagen deposition, measured by total hydroxyproline content, in the infarcted or noninfarcted areas in this study. It is possible that antiplatelet, anti-thrombotic, and spasmolytic effects of nitrates\textsuperscript{32,33} contributed in maintaining perfusion.

In this study, nitrates also prevented the increase in left ventricular mass found in the control group. As noted previously,\textsuperscript{25,31} there was no significant increase in postmortem left ventricular mass by 6 weeks after infarction in this model. However, in vivo left ventricular mass measured by serial echocardiography increased over the 6 weeks in the control group and decreased with nitrate therapy. More important, the effect was greater with long-term than short-term therapy.

A similar beneficial effect of prolonged nitrate therapy on left ventricular mass and volume was found in a recent study\textsuperscript{34} in which isosorbide mononitrate was given twice daily for 16 weeks after myocardial damage produced by transmyocardial DC shock in dogs. In that study,\textsuperscript{34} nitrate therapy in 10 dogs prevented both the increase in left ventricular mass and volume measured in a group of controls (4 randomized and 13 from a prior study) between 1 and 6 weeks using nuclear magnetic resonance. Also in that study,\textsuperscript{34} nitrate therapy was associated at 16 weeks with a decrease in pulmonary capillary wedge pressure but not mean aortic pressure, suggesting that the beneficial effects were mainly due to the decrease in preload, volume, and wall stress. However, most of the increase in mass in that study\textsuperscript{34} occurred by 1 week (68.1 to 80.1 g or 18%), with little further increase between 1 week and 16 weeks (80.1 to 80.8 g or 1%), and most of the increase in volume occurred between 1 and 16 weeks (58.0 to 70.0 mL or

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**Fig 5.** Line plots: Effect of nitrate on left ventricular (LV) dilation in vivo. Percent changes on echocardiograms at the papillary level over seven time intervals after infarction. NTG ST indicates short-term nitrate subgroups; NTG LT, long-term nitrate subgroup. * *, fP value for control vs NTG LT.

**Fig 6.** Line plots: Effect of nitrate on left ventricular (LV) dysfunction in vivo. Percent changes on echocardiograms at the papillary level over seven time intervals after infarction. NTG ST indicates short-term nitrate subgroups; NTG LT, long-term nitrate subgroup. * P value for control vs NTG ST and LT, **P value for control vs NTG LT only.

**Fig 7.** Bar graph: Effect of nitrate on left ventricular mass in vivo. C indicates control group; NTG, nitrate; ST, short-term; and LT, long-term. *, **P values between control and all NTG. P<.05 refers to the difference between ST and LT nitrate subgroups.
21%), with little change by 1 week (55.8 to 58.0 mL or 4%). The finding that nitrates prevented the increase in mass over the first week despite lack of significant differences in hemodynamic parameters and volumes between the groups during that time in that model suggested the possibility that nitrates might influence the growth of myocytes and/or interstitium via effects on trophic substances and prevent hypertrophy via a purely nonhemodynamic mechanism. However, this still remains to be proven. Recent experimental studies suggested that endogenous vasodilators and nitrates exert antiproliferative effects on several tissues in vitro. Although the finding of increased mass without volume enlargement by 1 week in the “current damage” model supports the notion that an increase in left ventricular mass after myocardial damage can occur without the stimulus of cavity enlargement, it is not clear why the late increase in volume was not associated with further increase in mass in the “current damage” model.

It should be noted that, in contrast with that study, the control group in our study showed significant increases in noninfarct wall thickness and left ventricular volume as mass increased by 1 week and that these increases persisted over the 6 weeks in the control group but were prevented in the nitrate group. Apart from the difference in the mode of myocardial damage between that study and ours, other pertinent differences in that study were that (1) hemodynamics were measured under sedation or anesthesia, and mass and volumes were measured under anesthesia, (2) these measurements were only made at baseline, 1 week, and 16 weeks, (3) randomization was to 10 treated and 4 control dogs plus 13 more no-treatment dogs from a previous study, (4) detailed, serial in vivo measurements of geometry and function were not made, (5) postmortem topography, morphometry, and collagen were not measured, and (6) short-term and long-term therapy targeted to phases of healing after injury were not compared.

Although chronic nitrate administration produces tolerance, all four nitrate regimens in this study were beneficial. Intermittent therapy (subgroups 1, 3, and 4) and sustained therapy (subgroup 2) successfully attenuated multiple parameters of remodeling. In a recent report, Pipilis et al found that 20 mg of isosorbide mononitrate in regular doses three times daily to patients after suspected myocardial infarction did not produce sustained hemodynamic effects. However, they did not measure remodeling parameters. In contrast, intermittent and eccentric nitrate dosing regimens were found to have sustained beneficial effects on remodeling parameters and function in preliminary reports of two randomized clinical studies. Although tolerance develops within an hour in vitro when large concentrations are used, the effects of nitrates are known to last much longer in patients in whom much lower concentrations are used. In this study, attenuation of the hemodynamic effects was detectable after 1 week but was only partial.

Timing and duration of nitrate therapy also appeared to significantly influence the magnitude of the benefits. Because healing after infarction is not completed for about 6 weeks in dogs and 3 to 6 months in humans, it is logical to propose that “antiremodeling” therapy would be expected to be potentially more beneficial if applied throughout the healing phase than if applied for only a fraction of that time. In addition, because infarct expansion occurs early and collagen deposition into the already-expanded segments tends to make the shape deformation permanent, it would seem logical to begin therapy before collagen deposition plateaus at about 2 weeks. In this study, both therapies for the first 2 weeks and 6 weeks were beneficial, but therapy over 6 weeks imparted greater benefits. In fact, there was a suggestion that discontinuation of therapy at 2 weeks might be followed by further remodeling. However, short-term and long-term therapy had nearly similar beneficial effects on reducing infarct thinning, the extent of asynergy, and the frequency of apical aneurysm.

Clinical Relevance
First, the results of this study indicate that further remodeling occurs after the infarction process is completed and that this can be attenuated by chronic nitrate therapy. Second, the results indicate that both early short-term and long-term therapies during postinfarct healing are beneficial but that the benefit is greater with therapy applied throughout healing. Third, the results provide rationale for the clinical application of prolonged nitrate therapy after infarction to limit remodeling and preserve function.

Several preliminary clinical studies have focused on the effects of prolonged nitrate therapy between 48 hours and 6 weeks after infarction and after low-dose intravenous nitroglycerin for the 48 hours with or without thrombolytic therapy. These studies also suggested beneficial effects on ventricular remodeling and function as well as clinical outcome. The recently completed ISIS-4 trial explored the effect of very early intravenous magnesium followed by isosorbide mononitrate and/or captopril on mortality in patients with suspected infarction. The preliminary results presented at the American Heart Association Meeting in November 1993 indicated that, in a heterogenous (some low-risk patients with inferior or no infarction) and highly selected group of patients, most of whom had already benefited from fibrinolytic and/or intravenous nitrate therapy and subsequently received other concurrent medications at the discretion of the responsible physician, the 35-day mortality in the placebo group was low (6.92% to 7.33%), and the decrease in mortality with oral isosorbide-5-mononitrate (60 mg qd for 28 days) was not statistically significant (7.22% versus 6.98%, P=NS). However, they also reported that the decrease in mortality with oral captopril (50 mg bid for 28 days) was significant (7.33% versus 6.87%, P=.04), and the increase in mortality with intravenous magnesium sulfate over the first 24 hours was not significant (6.92% versus 7.28%, P=NS). Effects on left ventricular remodeling were not studied. The beneficial effect of captopril on some postinfarction remodeling parameters has already been documented experimentally in the canine model and clinically. Because of proven antianginal effects, nitrates might also be expected to reduce postinfarction angina and associated cardiovascular events.

Conclusions
Prolonged nitrate therapy after anterior myocardial infarction is effective in limiting progressive topographic changes during remodeling and interrupts the vicious cycle of more expansion, thinning, ventricular dilation, and aneurysm formation in the dog model. Prolonged therapy throughout healing provides greater benefit
than an early pulse of therapy. Nitrate tolerance was minimized and was only partial with the eccentric dosing regimens that have been suggested to provide a daily nitrate-free interval. Further clinical studies are needed to assess the effect of chronic nitrate therapy on postinfarct survival.

Acknowledgments
This study was supported in part by grants from the Medical Research Council of Canada and the Canadian Heart and Stroke Foundation, Ottawa, Ontario. We acknowledge the assistance of the sonographers and research technicians with data collection, graduate students, Brad Johnston, Curtis Johnston, Michael Joljart, Joanne Jugdutt, and Ruwan Liyanage with data processing, and Dr Gordon Blinston, PhD, with statistics.

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Effect of prolonged nitrate therapy on left ventricular remodeling after canine acute myocardial infarction.
B I Jugdutt and M I Khan

Circulation. 1994;89:2297-2307
doi: 10.1161/01.CIR.89.5.2297
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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