DITPA stimulates arteriolar growth and modifies myocardial postinfarction remodeling

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DITPA stimulates arteriolar growth and modifies myocardial postinfarction remodeling. Am J Physiol Heart Circ Physiol 286: H1994–H2000, 2004; 10.1152/ajpheart.00991.2003.—Myocardial infarction (MI) is characterized by ventricular remodeling, hypertrophy of the surviving myocardium, and an insufficient angiogenic response. Thyroxine is a powerful stimulus for myocardial angiogenesis. Male rats that underwent coronary artery ligation and subsequent MI were given 3,5-diiodothyropropionic acid (DITPA; MI + DITPA group) during a 3-wk period. We evaluated ventricular remodeling using echocardiography and histology and myocardial vessel growth using image analysis. Protein expression was assessed using Western blotting and immunohistochemistry. This study tested the hypothesis that the thyroxine analog DITPA facilitates angiogenesis and influences postinfarction remodeling in the surviving hypertrophic myocardium. The increase in the region of akinesis (infarct expansion) was blunted in the MI + DITPA rats compared with the MI group (3 vs. 21%); the treated rats had smaller percent increases in the left ventricular (LV) volume (64 ± 14 vs. 95 ± 12) and the LV volume-to-mass ratio (47 ± 13 vs. 84 ± 10) as well as a blunted decrease in ejection fraction (−9 ± 8 vs. −30 ± 7%). Arteriolar length density was higher after treatment in the largest (>50% of the free wall) infarcts (64 ± 3 vs. 43 ± 7%). Angiogenic growth factors [vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)] and the angiopoietin receptor tyrosine kinase with immunoglobulin and epidermal growth factor homology domains (Tie-2) values were elevated during the first week after infarction. DITPA did not cause additional increases in VEGF or Tie-2 values but did induce an increase in bFGF value after 3 days of treatment. This study provides the first evidence for an anatomical basis, i.e., attenuated ventricular remodeling and arteriolar growth, for improved function attributed to DITPA therapy for an anatomical basis, i.e., attenuated ventricular remodeling and arteriolar growth, for improved function attributed to DITPA therapy in the MI + DITPA group. In conclusion, arteriolar growth, angiogenic growth factors, and vascular remodeling were increased in MI + DITPA rats compared with MI rats, suggesting that this thyroxine analog may be a novel therapeutic angiogenic agent. Further studies are needed to determine the mechanism of action of DITPA and its role in postinfarction angiogenesis.

METHODS

Animals and experimental protocols. Male Sprague-Dawley rats (body wt, 300–325 g) were used for all experiments. All procedures were approved by the University of Iowa Animal Care and Use Committee and were in accordance with the regulations of the Animal Welfare Act of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996). The rats were anesthetized with ketamine-HCl (50 mg/kg) and xylazine (5 mg/kg, ip). After the heart was externalized via an incision between the 4th and 5th intercostal spaces, the proximal left coronary artery was ligated. The heart was immediately internalized and the chest was closed. Approximately 70% of the rats survived the procedure. For sham-operated animals, the suture was placed but not ligated. One day after ligation, the animals were assigned to either a DITPA-treatment (MI + DITPA) or vehicle-injection (MI) group. DITPA was injected daily (3.5 mg/kg body wt sc) as previously described (33). This assignment was made after echocardiography, which enabled us to match the two groups relative to the sizes of the ischemic regions (Table 1).

Growth factor and receptor proteins were assayed 1, 3, and 7 days after infarction. All other data were obtained 3 wk after coronary artery ligation, at which time a second echocardiographic evaluation was performed. After this evaluation the rats were anesthetized, hemodynamics were evaluated, and the heart was arrested with

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Table 1. Echocardiographic data from rats 1 day after infarction

<table>
<thead>
<tr>
<th></th>
<th>MI</th>
<th>MI + DITPA</th>
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<tbody>
<tr>
<td>Ischemic region, % of left ventricle</td>
<td>45 ± 3</td>
<td>45 ± 2</td>
</tr>
<tr>
<td>LV volume/mass</td>
<td>0.73 ± 3</td>
<td>0.81 ± 4</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>0.44 ± 0.04</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>412 ± 12</td>
<td>418 ± 15</td>
</tr>
</tbody>
</table>

Values are means ± SE. The % ischemic region was the variable used to assign rats to the two groups. MI, myocardial infarction; DITPA, 3,5-diiodothyropropionic acid; LV, left ventricular.

lidocaine-HCl and preserved by retrograde perfusion fixation via the aorta. The ventricles were cut with a four-blade guillotine, each of the resulting 2-mm slices was photographed, and infarct size was determined by image analysis. The area of the scar region for each of the heart slices was demarcated along with the area of the total LV free wall. Infarct size is expressed as a percent of the LV free wall.

Ventricular hemodynamics. To characterize LV function, we measured arterial and ventricular pressures and LV developed pressure. Rats were anesthetized with ketamine-HCl (50 mg/kg), intubated, and artificially ventilated. A polyethylene-50 catheter filled with heparinized saline (50 U/ml) was then introduced into the right carotid artery and connected to a computerized data-acquisition system (PowerLab/4S with Chart software; ADInstruments) coupled with a pressure transducer (BP-100; iWorx; Dover, NH). After the baseline blood pressure value was recorded, the catheter was slowly advanced into the left ventricle via the aorta. A thoracotomy was then performed to visualize the aortic arch, and a piece of silk was passed underneath the vessel. Arterial and ventricular pressures were recorded, and isovolumic developed pressure was assessed by performing a brief ligation of the aorta. The heart, arrested in diastole with lidocaine, was placed on a Langendorff apparatus and perfusion fixed with a glutaraldehyde-paraformaldehyde solution as previously described (30, 33).

Echocardiography. The procedures briefly described here have been extensively published (7, 8). Rats were evaluated 24 h after coronary artery ligation and again 3 wk later. Ketamine-HCl (25 mg/kg) was used to induce a semiconscious state. Short- and long-axis images were acquired with an 8-MHz sector-array probe. The resultant high-quality 2-D images were obtained at a rate of 40 frames/s. LV volume and mass and connected to a computerized data-acquisition system (PowerLab/4S with Chart software; ADInstruments) coupled with a pressure transducer (BP-100; iWorx; Dover, NH). After the baseline blood pressure value was recorded, the catheter was slowly advanced into the left ventricle via the aorta. A thoracotomy was then performed to visualize the aortic arch, and a piece of silk was passed underneath the vessel. Arterial and ventricular pressures were recorded, and isovolumic developed pressure was assessed by performing a brief ligation of the aorta. The heart, arrested in diastole with lidocaine, was placed on a Langendorff apparatus and perfusion fixed with a glutaraldehyde-paraformaldehyde solution as previously described (30, 33).

Stereological analysis of angiogenesis. Specimens were dissected from either 1) a 1-mm region bordering the infarct, and 2) ventricular septum of perfusion-fixed hearts, and were processed and embedded in Spurr’s plastic. The 1-mm sections of the ventricular samples were cut perpendicular to the long axes of muscle fibers, placed on glass slides, and stained with azure II and methylene blue (Richardson’s solution). Image analysis of capillary and arteriolar profiles was performed using Image-Pro software (Media Cybernetics; Silver Spring, MD). The procedure for this stereological analysis, which is used routinely in our lab, has been detailed previously (27, 33). Vessel length density was used as an index of angiogenesis and is based on the following calculation: \( L_v = \frac{a b N_\Lambda}{b a} \), where \( a \) and \( b \) are the long and short axes, respectively, and \( N_\Lambda \) is the number of vessel profiles in the field (numerical density). Volume density (Vd) was determined directly by pixel density (i.e., number of pixels/lumen \( \times \) number of profiles). Arterioles were defined as vessels <50 \( \mu \)m in diameter that had at least one layer of smooth muscle; vessels with no smooth muscle and diameter <10 \( \mu \)m were noted as capillaries. A minimum of 70 arterioles and 350 capillaries were measured for each sample.

Cardiomyocyte length data. Digestion of glutaraldehyde-fixed myocardial tissue using potassium hydroxide (KOH) was performed as previously described (27). Briefly, sections of the septum and border region were cut and digested with 2% trypsin (Amresco; Solon, OH), followed by 50% KOH in PBS. Softened tissue was minced and filtered through 105- \( \mu \)m nylon mesh (Fisher; Pittsburgh, PA). The filtered cells were photographed, and cell length was measured using Image-Pro software. From subsamples of tissue specimens representing a variety of infarct sizes, ultrathin longitudinal sections adjacent to tissue samples from which myocytes were isolated were prepared with a diamond knife for electron microscopy. After sections were stained with uranyl acetate and lead citrate, they were examined with a Hitachi H-7000 electron microscope. These sections were used for the determination of sarcomere lengths. To adjust for plane of sectioning, we adjusted sarcomere values by measuring the A band and assigning it a value of 1.5 \( \mu \)m.

Western blot assay. Tissue samples from the border and septum region of 3 rats per group were homogenized in lysis buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, and 10 mM EDTA in PBS) that contained protease inhibitors, and 50 \( \mu \)g of proteins were separated by SDS-PAGE. The proteins were then transferred from a gel to a nitrocellulose membrane. The membranes were blotted with basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), tyrosine kinase with immunoglobulin and epidermal growth factor homology domains (Tie-2) antibodies (Santa Cruz Biotechnology; Santa Cruz, CA), angiopoietin-1 and -2 antibodies (Alpha Diagnostic International; San Antonio, TX) and GAPDH antibody (Chemicon; Temecula, CA). The antigen-antibody complexes were visualized by the appropriate secondary antibodies and chemiluminescence system (Pierce; Rockford, IL).

Histology and immunohistochemistry. Paraformaldehyde-fixed hearts were paraffin embedded, sectioned at a thickness of 6 \( \mu \)m, and affixed to Superfast Plus slides. Immunohistochemistry was performed as previously described (32). Slides were placed in a humidified chamber, and incubations were conducted with the primary antibody (bFGF and VEGF antibodies) and subsequently with secondary antibody (Chemicon). The slides were visualized after incubation in alkaline phosphatase substrate solution (Vector Laboratories). To assess cardiomyocyte cross-sectional area and general histology, slides were stained with the periodic acid methenamine-silver procedure.

Statistical analysis. Student’s t-test was used for one-way comparisons between MI and MI + DITPA groups, and ANOVA analysis and the Bonferroni post hoc test were employed for multiple comparisons. To test the effects of infarct and treatment over time on growth factors, we used the Helmert contrast t-values test.

RESULTS

Baseline ventricular function. On postinfarction day 1, there were no significant differences between the MI and MI + DITPA groups with respect to heart rate, LV function, and ischemic zone size (Table 1). At this time, ischemic region percent values were used to assign rats to each of the two groups. As seen in Table 1, the mean ischemic regions were identical in the two groups.

Ventricular remodeling and function. After 3 wk of DITPA treatment, no significant intergroup differences existed for ventricular wt/body wt, ventricular diastolic pressure, and systolic and diastolic arterial pressures; however, the developed pressure measurements were significantly higher (by 20%) in the MI + DITPA animals compared with the MI rats (Table 2).
The increase of the developed pressure implies that the DITPA treatment may improve contractile function (Table 2).

Figure 1 summarizes data that are indicative of attenuated ventricular remodeling in DITPA-treated rats. These echocardiographic data represent percent changes between 1 day after coronary artery ligation and 3 wk of DITPA therapy. The findings indicate that DITPA therapy attenuated the increase in ventricular volume by 35% and the increase in the ventricular volume-to-mass ratio by about half. Most important, DITPA reduced infarct expansion by ~80%, which indicates that preservation of contractile function in the ischemic border zone was limited by therapy. These positive effects on ventricular remodeling and infarct expansion were associated with a blunting of the postinfarction deterioration of ejection fraction (Fig. 1). The MI group experienced a 0.14 decline in ejection fraction compared to a negligible 0.01 drop in the MI+DITPA group. To determine the anatomical basis for the attenuated remodeling with treatment, we measured the lengths of isolated myocytes and myocyte cross-sectional areas in tissue samples. As seen in Fig. 2, the increases in cell length and cross-sectional area were similar for the MI and MI+DITPA groups. Mean increases in cell length for the MI and MI+DITPA groups were 35 and 33%, respectively, in the border region and 39 and 38%, respectively, in the septum. Myocyte thickness in the MI and MI+DITPA groups increased 67 and 58%, respectively, in the border region and 41 and 56%, respectively, in the septum. Moreover, sarcomere lengths, which were determined by electron microscopy, were virtually identical in the two groups (means ± SD: MI, 2.09 ± 0.09; MI+DITPA, 2.1 ± 0.03). Thus cell dimensions do not account for differences in remodeling between the two groups. Accordingly, the extent of myocyte side-to-side slippage, which was previously documented as a mechanism of remodeling (22), may have been less in the treated group. To address the potential contribution of direct inotropic effects of DITPA, we measured septal systolic wall thickening and found that systolic function was similar in the two groups.

Examination of histological slides stained with the silver-methanomine method for collagen did not reveal differences in this parameter in the spared myocardium. Regions not bordering the scar did not show evidence of fibrosis in either the MI or MI+DITPA groups.

Arteriolar growth. Stereological data regarding capillaries, which are provided in Table 3, indicate that DITPA therapy did not increase the growth of these vessels in the surviving myocardium as indicated by length density, volume density, and diameter measurements. Moreover, infarct size did not affect these values (data not shown). In contrast, arteriolar length density was significantly enhanced in the septums of rats with large infarcts, i.e., those >50% of the LV free wall (Fig. 3). Regression analysis of the septal data for all infarct sizes (20–80%) indicates an r value of 0.82 for infarct size and arteriolar density for the DITPA group (y = 1.08 – 8.36) compared to an r value of 0.37 for the nontreated group (y = 0.34 + 20.78). A trend toward higher values was also noted in the border region of DITPA-treated compared with nontreated rats. The formation of new arterioles in the hearts that experienced infarcts >50% is suggested by the frequency distributions of the arteriolar diameters, which indicate that arterioles ±15 μm, i.e., terminal arterioles, constitute 83% of the arteriolar hierarchy in the MI+DITPA group compared with 63% in the nontreated MI group.

Growth factors. To investigate the molecular mechanism involved in vascular growth or remodeling, we examined the expression of VEGF, bFGF, Tie-2, and angiopoietin-1 and -2 proteins with Western blot analysis (Fig. 4). The quantitative comparisons for these proteins are shown in Fig. 5. One day after infarction, the bFGF protein level was increased ~2.2-fold in the border region of the nontreated MI group; it then declined by day 3. However, in the DITPA-treated MI group, DITPA prolonged the bFGF upregulation for up to 3 days (P < 0.01). No significant increase in bFGF was found in the septum. Infarction resulted in three- to fourfold upregulation in VEGF protein in both the DITPA-treated and nontreated

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**Table 2. Heart mass and hemodynamic data from rats with MI after 3 weeks of DITPA therapy**

<table>
<thead>
<tr>
<th></th>
<th>MI</th>
<th>MI+DITPA</th>
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<tbody>
<tr>
<td>Ventricular wt/body wt</td>
<td>3.16±0.07 (21)</td>
<td>3.13±0.07 (22)</td>
</tr>
<tr>
<td>Septal wall thickness, mm</td>
<td>1.45±0.09 (13)</td>
<td>1.41±0.04 (14)</td>
</tr>
<tr>
<td>Infarct size, % of free wall</td>
<td>49.05±5.03 (22)</td>
<td>46.71±3.29 (20)</td>
</tr>
<tr>
<td>Systolic arterial pressure, mmHg</td>
<td>102±3 (22)</td>
<td>109±5 (14)</td>
</tr>
<tr>
<td>Diastolic arterial pressure, mmHg</td>
<td>70±3 (22)</td>
<td>78±4 (14)</td>
</tr>
<tr>
<td>Ventricular diastolic pressure, mmHg</td>
<td>9.71±3.0 (21)</td>
<td>8.70±2.18 (10)</td>
</tr>
<tr>
<td>Developed pressure, mmHg</td>
<td>189±8 (13)</td>
<td>227±8* (9)</td>
</tr>
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</table>

Values are means ± SE; n, no. of animals (in parentheses). LV wall thickness is mean thickness of surviving myocardium. *P < 0.02, statistically significant compared with MI alone.

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Fig. 1. Left ventricular (LV) echocardiographic changes over 21 days after myocardial infarction (MI). Rats that underwent MI and were subsequently given 3,5-diiodothyropropionic acid (MI+DITPA) are compared with untreated rats (MI). Means ± SE were derived from the differences between the first and second echocardiographs for each rat. Volume is that of the ventricular lumen, and mass includes the LV free wall and septum. Ischemic zone consists of the akinetic region. Number of rats, n, is shown in parentheses.
groups in the border region. The increase in the septum was much more modest. In contrast with bFGF, no significant change in VEGF protein was observed between DITPA-treated and nontreated groups. Furthermore, Western blot analysis demonstrated that Tie-2 values slightly increased in border and septum regions after infarction, but the increase was not enhanced further or prolonged by DITPA treatment. Angiopoietin-1 and -2 were upregulated in a time-dependent manner in the septum during the first week after MI. DITPA treatment did not increase these values.

**DISCUSSION**

This study provides new evidence that the thyroid analog DITPA facilitates arteriolar growth, modifies ventricular remodeling, and limits infarct expansion and the decline in ejection fraction in the postinfarcted heart. Our data show that DITPA therapy minimized the increase in the volume-to-mass ratio via a decrease in ventricular dilatation and markedly blunted infarct expansion. In large infarcts, DITPA increased arteriolar length density in the hypertrophic myocardium by 49%. These changes were associated with a higher value for developed pressure compared with the nontreated infarcted group.

Previous studies on DITPA therapy of infarcted hearts have documented attenuation of adverse functional changes (26). Initial studies showed that DITPA in combination with captopril increased the cardiac index in rats with large infarcts more than captopril alone. Subsequently, DITPA therapy alone in rabbits with infarction was found to attenuate ventricular end-diastolic pressure, improve LV relaxation, and increase positive and negative dP/dt values (17). Moreover, the frequency-dependent abnormalities of contractility, Ca2+ cycling, and action potential repolarization in single myocytes from infarcted hearts are largely prevented with chronic DITPA treatment (16). These data indicate improvement in the function of surviving myocytes. The data from the present study extend those earlier findings by documenting a favorable effect on postinfarct remodeling. Furthermore, we document arteriolar growth as a component of the remodeling process. Thus we provide evidence for two important anatomical adaptations attributed to DITPA therapy.

**Angiogenesis and arteriogenesis.** This study was precipitated by our recent documentation that in noninfarcted rat hearts, DITPA increases 1) VEGF164, VEGF188, FGF-2 (bFGF), angiopoietin-1, and Tie-2 during the first few days of treatment; and 2) arteriolar length density and the number of terminal arterioles after 3 wk of treatment (36). Although our current data concerning therapy on rats with large infarcts mimics the arteriolar growth in noninfarcted hearts, the role of growth factors as regulators of this angiogenesis is less clear. During the first week of infarction, bFGF, VEGF, and Tie-2 levels (to a lesser degree) were elevated. DITPA did not cause an additional increase in these growth factors with the exception of bFGF, which was higher in the border region after 3 days of treatment. Thus bFGF may have played the key role in this response. Alternatively, other growth factors in the treated group may have remained elevated for a longer period of time (after the first week) in the DITPA group. Importantly, this effect is dependent on the infarct size, because the arteriolar growth was limited to the surviving myocardium of hearts with infarcts >50% of the LV free wall.

The mechanism by which DITPA induced arteriolar growth is not clear. There are, however, several effects of DITPA that may have facilitated this growth. These include decreases in isovolumetric relaxation time and systemic vascular resistance (20) and a faster time to peak shortening rate (16). These effects would serve to decrease the time that myocardial compressive forces impinge on coronary transmural forces. Accordingly, the time period that arterioles are in a relatively open configuration is greater. This situation could provide a

**Table 3. Stereological capillary data from DITPA-treated and nontreated rats with 3-wk-old myocardial infarcts**

<table>
<thead>
<tr>
<th>Capillary parameter</th>
<th>MI</th>
<th>MI + DITPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Border</td>
<td>Septum</td>
</tr>
<tr>
<td>Length density, mm/mm³</td>
<td>6.695±217</td>
<td>8.253±299</td>
</tr>
<tr>
<td>Volume density, mm³/mm³×100</td>
<td>11.06±0.35</td>
<td>12.09±0.31</td>
</tr>
<tr>
<td>Diameter, μm</td>
<td>4.41±0.10</td>
<td>4.12±0.10</td>
</tr>
</tbody>
</table>

Values are means ± SE. No. of observations: MI, 22; MI + DITPA, 16. None of the differences between the two groups is statistically significant.
In the study, we found a significant correlation between infarction and the treatment with DITPA for 10 days (33). In this scenario, we documented higher capillary length density values in rats treated with DITPA. This result is consistent with an earlier study that reported higher values of capillary length density after 10 days of growth in that study, however, it noted a trend toward noninfarcted DITPA-treated rats (36). Some capillary infarcted rats is consistent with our previous study on density values were not higher 21 days after treatment of the nontreated rats. This is evident from the data that document 71 and 33% larger cardiomyocytes cross-sectional areas in the infarct group compared with the shams. Because arteriolar length density measurements were similar to values for control rats documented in our earlier study (36), arteriolar growth approximately paralleled the myocardial hypertrophy. The mechanism(s) underlying the greater arteriolar growth with DITPA treatment in rats with large infarcts is not understood at this time. Possible explanations might include prolongation of the increased levels of growth factor proteins or activation of other growth factors, cytokines, or physical factors unique to the treatment group. Another possibility is that large infarcts cause a greater degree of stretch on surviving myocytes, which facilitates the effects of DITPA. The finding that the capillary length density values were not higher 21 days after treatment of the infarcted rats is consistent with our previous study on noninfarcted DITPA-treated rats (36). Some capillary growth in this study, however, was noted by a trend toward higher values of capillary length density after 10 days of treatment. This is consistent with an earlier study that documented higher capillary length density values in rats with infarction that were DIPTA treated for 10 days (33). In that study, we found a significant correlation between infarct size and capillary length density in the border region. This finding is in concert with our current data that support the conclusion that arteriolar growth in DITPA-treated rats is greatest in large infarcts.

**Ventricular remodeling.** LV dilatation after MI is associated with depressed ventricular function and high mortality in patients and experimental animals (4). Accordingly, our novel findings that DITPA not only facilitates arteriolar growth but also minimizes both remodeling and infarct expansion is of clinical importance. Our data are based on pre- and post-treatment echocardiographs, which permit evaluation of changes in each rat over the 21-day period. An earlier study found that postmortem LV length and diameter measurements were similar in DITPA-treated and nontreated rabbits with MI (17). However, the influence of DITPA on remodeling could not be determined, because measurements were made only at the end of the study. The mechanism by which DITPA modifies the remodeling processes warrants further investigation. Infarct expansion is likely due to ischemia and/or apoptosis in the border region. One possible explanation for limitation of this process by DITPA therapy is an improved myocardial perfusion in this peri-infarction region. The data in Fig. 3 indicate significant enhancement of vascular growth by DITPA in the hypertrophied noninfarcted myocardium (septum). A priori, there appears to be a more modest effect of DITPA on vascular growth in the ischemic border zone. However, it is critically important to understand that in the postinfarction left ventricle, the “border zone” is dynamic during the remodeling process. It is likely that border-zone viability depends on maintenance or restoration of a minimum level of perfusion below which myocardial necrosis and/or apoptosis ensues. Thus it is not surprising that arteriolar densities were similar between treatment groups within the respective border zones, which are by definition viable. The echocardiographic data indicate that DITPA significantly limits infarct expansion. Phrased alternatively: DITPA preserves border zone viability. Thus the significance of DITPA-induced vascular growth is...
reflected by the anatomic extent of vascular growth and consequent border zone preservation rather than by the arteriolar density itself.

Although the increase in myocyte length that occurs with postinfarction chamber dilatation correlates with the severity of chamber geometry (1), the differences between our groups cannot be explained by this cell dimension, because both groups had similar lengths and sarcomere lengths did not differ. Both apoptosis and necrotic cell death occur in the postinfarcted heart (3) and therefore contribute to the side-to-side slippage of cardiomyocytes (22). We submit that less myocyte slippage may underlie the blunted chamber dilation in the DITPA-treated group. One possible mechanism limiting this ventricular dilatation is inhibition of metalloproteinases as documented by a study in mice with infarctions (25). Two-dimensional echocardiography revealed smaller end-systolic and -diastolic dimensions in the group treated with a broad-spectrum matrix metalloproteinase inhibitor compared with the nontreated MI group. The present studies in our laboratory are addressing this possibility.

**Limitations of study.** We report an association between improved arteriogenesis and attenuated infarct size expansion in the MI+DITPA group. The causal relationship between these two outcomes cannot be proven by our study design. However, the contention that DITPA treatment favorably influences postinfarction remodeling is supported by both observations. Echocardiographic depiction of the process of eccentric LV remodeling is imperfect. The major resulting fault would presumably be that the technique would not be sufficiently sensitive to detect small differences between groups. In the present study, the effects of DITPA treatment were of sufficient magnitude so as to be detected with high statistical significance using the echocardiographic methods. Moreover, ventricular volumes are strong independent predictors of survival after MI (37). Thus our study supports the therapeutic value of DITPA.

In conclusion, our data provide new evidence for two major benefits of DITPA therapy for the infarcted heart: 1) arteriolar growth enhancement; and 2) attenuation of LV remodeling resulting in a smaller increase in volume-to-mass ratio, a smaller infarct expansion, a higher developed pressure, and blunting of the decline in ejection fraction. These findings extend previous documentation of the favorable effects of DITPA on contractility including restoration of repolarizing transient outward K⁺ currents after MI (38) and curtailment of the downregulation of sarcoplasmic reticulum proteins associated with heart failure (24). Thus our findings expand the rationale for the therapeutic potential of DITPA.

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