EXPERIMENTAL STUDIES

Vascular Remodeling During Healing After Myocardial Infarction in the Dog Model

Effects of Reperfusion, Amlodipine and Enalapril

Bodh I. Jugdutt, MD, FACC,* Vijayan Menon, BSc,* Dinender Kumar, PhD,* Halliday Idikio, MD†

Edmonton, Alberta, Canada

OBJECTIVES

We sought to determine whether reperfusion and the calcium channel blocker amlodipine or the angiotensin-converting enzyme inhibitor enalapril, during healing over six weeks after myocardial infarction (MI), limit structural vascular remodeling in the noninfarct zone (NIZ).

BACKGROUND

The effect of reperfusion and amlodipine or enalapril on structural vascular remodeling during healing of MI has not been determined.

METHODS

We randomly assigned 54 dogs to reperfused or nonreperfused MI, followed by twice-daily doses of oral placebo, amlodipine (5 mg) or enalapril (5 mg) for six weeks and three days off treatment, or to three matching sham groups. We measured in vivo hemodynamic data and left ventricular (LV) function and remodeling (by echocardiography) over the six weeks, as well as ex vivo structural vascular, ventricular and collagen remodeling in the hearts after six weeks.

RESULTS

Compared with placebo and sham groups, both amlodipine and enalapril with or without reperfusion produced LV unloading and limited structural LV remodeling and dysfunction over six weeks in vivo, and also decreased the NIZ resistance vessel media/lumen area ratio at six weeks ex vivo. In addition, amlodipine, but not enalapril, preserved infarct scar collagen and increased the border zone collagen volume fraction and perivascular fibrosis, as well as NIZ resistance vessel media thickness. Enalapril, but not amlodipine, decreased transforming growth factor-beta in the border zone and NIZ.

CONCLUSIONS

The results indicate that therapy with amlodipine and enalapril during healing after reperfused MI limits structural vascular remodeling in the NIZ, probably by different mechanisms. (J Am Coll Cardiol 2002;39:1538–45) © 2002 by the American College of Cardiology Foundation

We hypothesized that therapy applied to limit structural left ventricular (LV) remodeling during healing after reperfused myocardial infarction (MI) (1) should modify structural vascular remodeling (2,3) in the noninfarct zone (NIZ). During healing after MI, increased collagen in the infarct zone (IZ) (1,4,5) and NIZ (1,5), together with NIZ hypertrophy, contributes to decreased LV distensibility (6) and diastolic dysfunction. Left ventricular hypertrophy in hypertensive patients is associated with an increased arteriolar wall thickness area, peri-arteriolar fibrosis, interstitial fibrosis and impaired coronary reserve (7). Coronary reperfusion after MI limits LV structural remodeling (8), but results in "reperfusion injury" (9), less IZ collagen (8), reduced nitric oxide release and coronary flow reserve (10). Angiotensin-converting enzyme (ACE) inhibitors produce many clinical benefits and limit LV dilation after MI (11), and also decrease collagen in the IZ (11) and NIZ (12) and in resistance arterioles in hypertensive patients (13,14). The new calcium channel blocker amlodipine improves survival in patients with nonischemic cardiomyopathy (15), limits LV dilation after infarction in dogs (16), rats (17) and cardiomyopathic hamsters (18). Both amlodipine and ACE inhibitors modulate kinin-mediated nitric oxide production in canine (19) and human (20) coronary microvessels, and also decrease the media thickness of resistance arteries in spontaneously hypertensive rats (13,14). Prolonged treatment of post-infarction rats with amlodipine induces vascular changes in the NIZ and improves the survival of rats with small infarcts (21). The aim of this study was to determine whether reperfusion and amlodipine or enalapril, during healing over six weeks after MI, limit structural NIZ vascular remodeling in association with LV structural and collagen remodeling in the dog.

METHODS

Experimental preparation. The studies were approved by the institutional Animal Welfare Committee and conformed with the “Position of the American Heart Association on Research Animal Use,” adopted by the Association in November 1984. Fifty-six healthy mongrel dogs (weight 17 to 22 kg) were instrumented through a left lateral thoracotomy, under general anesthesia (sodium pentobarbital, 30 mg/kg intravenously), as described previously (8).
Briefly, indwelling catheters were inserted in the external jugular vein, internal carotid artery and left atrium for monitoring hemodynamic variables. The mid-left anterior descending coronary artery was tied with a silk ligature. Visible epicardial feeders at the margins of the occluded bed were ligated to produce transmural injury (15). Metal beads were sutured on the epicardium of the mid-LV wall for consistent orientation of two-dimensional echocardiograms. Reperfusion (n = 19) was produced by removing the ligature 2 h after coronary occlusion. No ligations were made in 18 animals (sham group). The pericardium and chest were closed. Penicillin (1 million U) and streptomycin (1 g) were then given intramuscularly.

**Protocol.** Twenty-four hours later (day 2), 54 surviving animals with or without reperfused MI (one dog in each arm died) were randomized (2 x 3 factorial design) to three treatment groups (n = 6 per cell)—twice-daily oral placebo, amlodipine (Pfizer Canada Inc., Dorval, Quebec; 5-mg tablet) or enalapril (Merck Frosst Inc., Dorval, Quebec; 5-mg tablet) for six weeks—and three matching sham groups (n = 3 per cell). The doses of amlodipine and enalapril were previously confirmed to decrease the mean blood pressure by 10% at 4 to 6 h after oral administration on the second day after MI (16). After six weeks of therapy and three days off therapy, the animals were re-anesthetized and a thoracotomy was performed, and the hearts were arrested in diastole (1 mol/l of potassium chloride intravenously), quickly excised, washed in saline solution and fixed whole heart and radiographs of the formalinixed whole heart and five equally spaced transverse sections (1- to 1.5-cm thick). Fixation involved suspension in 10% phosphate-buffered formalin, 10 cm below the surface for 48 h. Outlines of LV rings, risk regions and infarct scars were made on plastic overlays and planimetered (Hewlett-Packard Vectra 486/33T computer; Summagraphics-Summasketch III) for infarct scar size and topographic variables, including the thinning ratio and expansion index (6,8,16).

**Ex vivo measurement of scar size and LV geometry.** As described previously (6,8,16), the risk regions were measured from postmortem coronary arteriograms of fresh hearts (using simultaneous injection into each coronary artery with a barium sulfate and gelatin mixture, under constant pressure of 160 mm Hg) and were recorded on radiographs of the formalin-fixed whole heart and five equally spaced transverse sections (1 to 1.5-cm thick). Fixation involved suspension in 10% phosphate-buffered formalin, 10 cm below the surface for 48 h. Outlines of LV rings, risk regions and infarct scars were made on plastic overlays and planimetered (Hewlett-Packard Vectra 486/33T computer; Summagraphics-Summasketch III) for infarct scar size and topographic variables, including the thinning ratio and expansion index (6,8,16).

**Measurement of collagen content and collagen volume fraction (CVF).** Myocardial hydroxyproline (mg/g dry tissue weight), a marker of collagen protein, was measured, as described previously (4,8,11), in 100-mg tissue samples from the center of the IZ (occluded bed) and NIZ (left circumflex territory) in the third LV ring. For CVF, samples from the IZ, NIZ, spared zone within the risk region and border zone outside the risk area of the fourth LV ring (Fig. 1) were embedded and sectioned, as described previously (22). Sections (4-µm thick) were stained with 1% picrosirius red for fibrillar collagen (23) and examined under a Nikon (Tokyo, Japan) Labophot II microscope (x200 magnification) fitted with cross-polarization filters and a high-
resolution digital color camera (Sanyo VCC-3770, Tokyo, Japan). Perivascular and interstitial collagen appeared red, and the remaining tissue appeared yellow. Interstitial collagen density was analyzed (Leco 2001 image analyzer, Longueuil, Quebec) by excluding perivascular fibrosis (PVF) and infarct scar, and was expressed as percent myocardial area.

**Assessment of vascular remodeling.** Sections from the IZ, NIZ and border and spared zones of the fourth LV ring were examined in blinded fashion by an experienced observer. Perivascular fibrosis was scored 1 to 5 semiquantitatively on sets of coded slides stained with picrosirius red or trichrome, and the average of the two scores was calculated. Planimetric analysis of blood vessels on the picrosirius-red slides was carried out using camera-produced Lucida drawings (×130, ×260 and ×520 magnifications) that were digitized on a graphics tablet (Summagraphics-Summasketch III) for the area and perimeters of the vessel wall and lumen, as well as the wall/lumen ratio. Average values were computed for each section. Only blood vessels with area 5 to 20 × 10^4 μm² and a form factor—computed using the formula (24): \( F = 4\pi \times A/P^2 \), where A = lumen area and P = perimeter—of F > 0.75 were included. The media/lumen ratio was plotted against the total blood vessel area, using a nonlinear curve fitter or power regression algorithm (Jandel Scientific Inc., San Rafael, California). Photographs were taken and scanned (Digital Science Photo CD software, Eastman Kodak Co., Rochester, New York).

**Transforming growth factor-beta (TGF-beta).** To determine whether changes in TGF-beta might explain the changes in collagen and PVF, histologic tissue sections (5 μm) from the NIZ, IZ and border zone of the hearts from the placebo, enalapril and amlodipine groups were immunostained by Dr. Kumar using the cell and tissue staining indirect immunoperoxidase kit (R&D Systems, Minneapolis, Minnesota), according to the manufacturer’s instructions (25), with primary polyclonal pan-specific rabbit antibody and secondary biotinylated anti-rabbit antibody. The sections were analyzed by Dr. Idikio, using a microscope (Olympus BH-2), and TGF-beta staining was scored from 0 to 3+.

**Statistics.** Data were analyzed in blinded manner, using analysis of variance with repeated measures and multiple comparisons (Student-Neuman–Keuls test) for the significance of differences within and between groups and for comparing serial data within groups. Results are presented as the mean value ± SEM. Statistical significance was set at p < 0.05.

**RESULTS**

**In vivo hemodynamic data and LV function and remodeling.** At baseline (day 2), all variables were similar in the sham and infarct groups, and, compared with the sham group, the infarct groups showed increased left atrial pressure and clearly different LV function and geometry. With amlodipine and enalapril with or without reperfusion over six weeks, the heart rate, mean arterial pressure, left atrial pressure and rate-pressure product all decreased, compared with baseline values (p < 0.05 to 0.001), and left atrial pressure persistently decreased (p < 0.001) three days after the active drugs were stopped, compared with placebo. Compared with the nonreperfused placebo group six weeks after MI, total LV asynery increased in all reperfusion groups (−33% vs. 16%, p < 0.05); the thinning ratio was lower in all treatment groups (−59% vs. −37%, p < 0.05) and actually increased in the nonreperfused amlodipine group (−59% vs. 10%, p < 0.001); the expansion index increased slightly in the reperfused enalapril and amlodipine groups (2.4% vs. 13%, p < 0.05); the diastolic and systolic volumes decreased in all treatment groups (p < 0.05 to 0.01); the global LV ejection fraction improved in the reperfused placebo group (6% vs. 18%, p < 0.01) and in the nonreperfused enalapril and amlodipine groups (6% vs. 32%, p < 0.01); the LV mass decreased in all treatment groups (24% vs. 2%, p < 0.001); and both the global shape index (8% vs. −2%, p < 0.005) and regional IZ bulging (86% vs. −7%, p < 0.02) decreased with amlodipine.

**Infarct scar size and collagen protein.** At six weeks, the infarct scar size in the nonreperfused placebo group (30 ± 2% risk region) was larger than that in the reperfused placebo (16 ± 4%, p < 0.05) and enalapril (15 ± 2%, p < 0.05) groups or in the nonreperfused amlodipine (18 ± 4%, p < 0.05) and enalapril (13 ± 3%, p < 0.001) groups. The hydroxyproline content (mg/g) in the IZ decreased from 45 ± 8 in the nonreperfused placebo group to 27 ± 6 in the reperfused group (p < 0.05), to 21 ± 4 in the amlodipine group with or without reperfusion (p < 0.01), to 9 ± 2 in the enalapril group with or without reperfusion (p < 0.002). Compared with the sham group, NIZ hydroxyproline (mg/g) increased from 3.6 ± 0.3 to 6.4 ± 1.0 in the placebo group (p < 0.007) and to 4.9 ± 0.5 in the amlodipine group (p < 0.03), but not in the enalapril group (4.1 ± 0.7).

**Collagen volume fraction (Figs. 2A and 2B).** Compared with the sham group, interstitial CVF increased significantly in the spared zone of the nonreperfused groups; the increase was most pronounced with amlodipine. Compared with placebo, CVF showed a mild increase in the border zone of the reperfused amlodipine group.

**Perivascular collagen (Figs. 2C and 2D).** Compared with the placebo and enalapril groups, amlodipine increased PVF in the nonreperfused spared zone and the reperfused border zone. Amlodipine also increased PVF (p < 0.05) in the noninfarcted interventricular septum and remote right ventricular regions in the reperfused and nonreperfused groups (data not shown).

**Vascular remodeling in the NIZ (Figs. 2E and 2F).** Data on the media/lumen ratio and media thickness for the reperfused and nonreperfused groups were pooled, as they were similar. Compared with the sham group, the media/lumen ratio increased in the placebo group and normalized in the spared and border zones and NIZ in the amlodipine
Figure 2. Effect of treatments on CVF (A, B), PVF (C, D) and the media/lumen ratio and media thickness (E, F). Data on vessels with areas 5 to 20 × 10^4 μm² are shown and pooled for hearts with or without reperfusion (E, F).
and enalapril groups (Fig. 2E), and the media thickness increased in the spared and border zones and NIZ in all treatment groups (Fig. 2F). The NIZ media thickness with amlodipine was greater than that with placebo and enalapril (Fig. 2F).

**Vessel size.** Power function plots confirmed that an increased media/lumen ratio was mainly related to remodeling of small vessels, with an area <20 × 10⁴ μm², and the regressions were slightly lower (p = NS) in the sham, enalapril and amlodipine groups than in the placebo group (Fig. 3). The increased ratio with placebo was due to an increase in the area of the media and a mild decrease in the lumen size, whereas the decreased ratio with amlodipine was associated with an increase in lumen size and media thickness (Fig. 4). The decreased ratio with enalapril was associated with a decrease in media thickness, compared with placebo, but was similar, relative to lumen size, to that with amlodipine (Fig. 5).

**Transforming growth factor-beta.** Compared with the placebo group, the TGF-beta score in the NIZ myocardial and perivascular regions decreased with enalapril (0.3 vs. 1.5), but not with amlodipine (2.3 vs. 1.5). Staining of TGF-beta was found mainly in cardiomyocytes, vascular smooth muscle cells and fibroblasts.

**DISCUSSION**

The results of this study indicate that significant remodeling occurs in resistance vessels of the NIZ in healed reperfused and nonreperfused hearts, with an increase in the media thickness and media/lumen ratio of the remote NIZ and border zone. Importantly, enalapril and amlodipine both normalize the media/lumen ratio in resistance vessels of the NIZ. However, this effect seems to involve different mechanisms, as the media thickness of the vessels in the NIZ increased with amlodipine, but not with enalapril. In addition, TGF-beta in the NIZ decreased with enalapril, but not with amlodipine.

**Importance of NIZ vascular remodeling.** To the best of our knowledge, structural vascular remodeling in the NIZ during healing after reperfused infarction, or its limitation by therapy, has not been reported previously. Studies of rats...
have shown that the NIZ undergoes hypertrophy, which is associated with decreased coronary flow reserve (26). The hypertrophy triggered by MI is most intense at the border zone and about a week after infarction, while vascular growth lags behind (2). The stimulus may be provided by neurohormonal activation (27) and release of angiotensin II (28), catecholamines, endothelin, fibrogenic growth factors such as TGF-beta, cytokines and other active molecules. Healed and remodeled post-infarction rat hearts show an impaired vascular reserve in the hypertrophied NIZ and a perfusion deficit in the border zone (2). The hypertrophied NIZ is therefore considered to be at increased risk of ischemic injury under stress, so that protective therapy might be desirable.

In this study, we found a quantitatively significant and similar increase in the media/lumen ratio and media thickness of the resistance arteries in the NIZ of both reperfused and nonreperfused placebo-treated hearts, compared with the sham group. Although we did not assess the functional significance of the structural changes under stress, these changes might explain the impaired vascular reserve reported in the NIZ (26). Although the vascular remodeling paradigm in LV hypertrophy associated with hypertension is also “increased wall thickness/lumen diameter (or wall/lumen) ratio,” it was suggested that there was a rearrangement of the existing material around a smaller lumen, without much growth (29). In contrast, growth plays a major role in the remodeling of cardiac vessels, ventricles and collagen matrix during post-infarction healing. Our finding of increased TGF in the NIZ of control animals, as well as its attenuation by enalapril (but not amlodipine), suggests the observed changes in media thickness, PVF and interstitial fibrosis may involve angiotensin II and TGF-beta. Further studies of TGF-beta isoforms, cell types and signaling and the possible role of the kinin–nitric oxide pathway are needed to clarify the precise mechanism.

Limitations of NIZ vascular remodeling. One goal of treatment of structural vascular remodeling is normalization of the vascular structure (29). In this study, six weeks of therapy with amlodipine or enalapril normalized the media/lumen ratio in the NIZ resistance vessels, compared with the sham group. This vascular effect was associated with previously reported, overall beneficial effects of reperfusion (8), ACE inhibition (11) and amlodipine (16) on LV structural remodeling and dysfunction, suggesting that it might have contributed to these benefits. However, the effects of amlodipine and enalapril on different variables of both LV and vascular remodeling after reperfusion were complex. Importantly, the decrease in the media/lumen ratio in the NIZ was not accompanied by a decrease in media thickness. In fact, media thickness in NIZ vessels 1) remained increased in all treatment groups, compared with sham; 2) was similar with enalapril and placebo; and 3) was actually greater with amlodipine, compared with placebo, enalapril and sham. The underlying explanation was that the lumen size showed a relatively mild increase with enalapril and a relatively more marked increase with amlodipine (Fig. 5). We are not aware of other reports on the limitation of remodeling of small resistance arteries in the

Figure 4. Typical photomicrographs of noninfarct tissue six weeks after myocardial infarction. Picrosirius-red staining shows collagen as red, and myocytes and other structures as yellow. A1, B1 = polarized light showing collagen as bright yellow or green; A2, B2 = placebo effect; B1, B2 = effect of amlodipine, showing a resistance vessel with an increased area of media, relative to the lumen, and perivascular fibrosis. Arrows indicate vessels. Crystalline material from postmortem arteriograms are seen within the lumen.

Figure 5. Diagram showing changes in the resistance vessel lumen (inner circle) and wall (area between inner and outer circles). The dark area represents perivascular fibrosis. The wall/lumen ratio is nearly similar for enalapril and amlodipine, although the wall area is less with enalapril and greater with amlodipine, compared with placebo.
NIZ by an ACE inhibitor or calcium channel blocker after MI. Previous studies. In hypertensive patients, an increased intramyocardial vessel wall area and PVF are associated with impaired vasodilatory reserve (7), and the ACE inhibitor cilazapril, administered over two years, decreases the media/lumen ratio of resistance arteries in eutectic fat biopsies. In spontaneously hypertensive rats, the ACE inhibitor perindopril produces dose-dependent regression of resistance coronary artery remodeling (30) and enalapril (10 mg/kg) or amlodipine (10 to 20 mg/kg) for 12 weeks produce regression of resistance coronary artery remodeling and a decrease in the media/lumen ratio (13). Because angiotensin II regulates cytosolic calcium in vascular smooth muscle, Sharifi et al. (13) speculated that enalapril and amlodipine might exert similar effects on vascular remodeling. In our infarcted hearts, we also found that amlodipine and enalapril decreased the media/lumen ratio, but amlodipine increased the media thickness of resistance arteries in the NIZ, although enalapril did not.

In post-infarction rats treated with amlodipine (5 mg/kg) for nine months, Whittaker et al. (21) found a similar increase in NIZ resistance coronary artery media thickness and PVF, as in our study, and speculated that the amlodipine-induced increase in media thickness and PVF might resist vessel compression and maintain flow (21). However, neither they (21) nor we measured regional coronary blood flow or vascular reserve with stress, so that the true functional significance of the structural resistance vessel remodeling in the NIZ remains to be studied. At rest, no differences in NIZ flow (2) or total LV flow (3) were detected in rat hearts after infarction. Whittaker’s (21) findings of a decreased lumen diameter, increased media/lumen ratio, no change in NIZ collagen and myocyte cross-sectional area differ from ours, but this might be related to the dose and longer exposure to amlodipine in their study, as well as differences in the severity of remodeling between rats and dogs (5). Using a higher dose of amlodipine (10 mg/kg) in post-infarction rats, Shimada et al. (17) showed attenuation of both the increase in LV mass and NIZ collagen messenger ribonucleic acid, as suggested in our study. It is possible that regional differences in the intensity of the growth response exist between the rat and dog models.

In support of our findings, amlodipine and enalapril have prevented LV remodeling and NIZ fibrosis in cardiomyopathic hamsters (18); amlodipine (31) and ACE inhibitor therapy (12) have decreased NIZ fibrosis in post-infarction rats; and angiotensin II type 1 receptor blockade (32), but not amlodipine (33), has decreased TGF-beta1 in kidney models.

A pertinent technical feature of our study is that all hearts were arrested in diastole and had postmortem arteriograms and fixation in distention. This systematic approach preserved not only the diastolic proportions of the chambers, but also the vessel shape (preventing collapse and distortion) during formalin fixation. In addition, all drug treatments were stopped three days before the hearts were removed, so as to minimize the possibility of persistent pharmacologic effects on vascular tone.

Conclusions. In this study, therapy with reperfusion followed by enalapril or amlodipine for six weeks after infarction limited structural vascular remodeling in the NIZ, with a decrease in the resistance vessel media/lumen ratio. This effect was associated with overall beneficial effects on structural LV remodeling and function, and may be clinically important. The functional significance of the vascular changes in the NIZ and whether they contributed to the limitation of ventricular remodeling and dysfunction with enalapril and amlodipine need further study.

Acknowledgments
We are grateful to the research assistants for their help with the animal model and data acquisition, and to Catherine Jugdutt for typing.

Reprint requests and correspondence: Dr. Bodh I. Jugdutt, 2C2.43 Walter Mackenzie Health Sciences Centre, Division of Cardiology, University of Alberta, Edmonton, Alberta, Canada T6G 2R7. E-mail: bjjugdutt@ualberta.ca.

REFERENCES


