Beneficial effect of myocardial angiogenesis on cardiac remodeling process by amlodipine and MCI-154

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Kumamoto, Hideki, Hiroshi Okamoto, Masashi Watanabe, Hisao Onozuka, Keiji Yoneya, Izumi Nakagawa, Satoru Chiba, Satoshi Watanabe, Taisei Mikami, Kazuhiro Abe, and Akira Kitabatake. Beneficial effect of myocardial angiogenesis on cardiac remodeling process by amlodipine and MCI-154. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1117–H1123, 1999.—The present study examined the effect of long-term treatment with amlodipine and MCI-154 (a Ca2+ sensitizer) on progressive cardiac dysfunction and microvasculature in the dilated cardiomyopathic (DCM) hamster heart. After treatment of DCM hamsters (Bio 53.58) with amlodipine or MCI-154 for 15 wk from the age of 5 wk, amlodipine and MCI-154 were found to cause an increase in left ventricular percent fractional shortening and decreases in left ventricular diastolic dimension and isovolumic relaxation time in echocardiograms (P < 0.01). A hemodynamic study showed that the diastolic time constant decreased in the amlodipine-treatment group (P < 0.05). In a morphometric study employing a double-staining method that discriminated arteriolar and venular capillaries, amlodipine and MCI-154 caused increases in total capillary density (P < 0.05) and the proportion of venular capillaries (P < 0.05). Moreover, Northern blot analysis showed that the expression of mRNA for vascular endothelial growth factor was significantly increased by amlodipine and MCI-154. They preserve coronary microvasculature in the DCM hamster and might induce angiogenesis of small vessels, thereby contributing to preservation of cardiac systolic and diastolic function.

THE ETIOLOGY AND PATHOGENESIS of idiopathic dilated cardiomyopathy (DCM) still have not been elucidated. The Syrian hamster with genetic cardiomyopathy is a reproducible, gradually progressive model of congestive heart failure resembling DCM in humans. The Bio 53.58 strain of experimental animals presents common DCM and develops heart failure at an early age. In this model, the pathogenesis of the cardiomyopathy is still unclear. One study suggested that focal and transient microvascular spasms cause focal myocarditis and ischemia (6) and that repeated reperfusion causes extended myocytolytic lesions equivalent to those observed in cardiomyopathy. In this model, calcium channel antagonists are very effective in slowing the progression of the disease and in reducing its severity (6, 17). Amlodipine, a third-generation, long-acting calcium channel antagonist, was reported to improve the mortality of patients with nonischemic DCM (16), and we previously reported that it prevents left ventricular (LV) remodeling and improves the cardiac systolic function in the DCM hamster (26). The mechanisms by which it acts in this model may be the prevention of microvascular spasms, improvement of the calcium metabolism of cardiac myocytes, and reduction of the afterload of the LV.

MCI-154, a newly developed Ca2+ sensitizer, is a positive inotropic agent that increases the Ca2+ sensitivity of the contractile apparatus. Ca2+ sensitization increases myocardial contractility by improving energy utilization of the myocardium without an increase in the intracellular concentration of cAMP. This compound has been shown to exert instantaneous inotropic effects on cardiac performance in heart failure (1, 24). However, it is not known how long-term treatment with MCI-154 affects cardiac function in DCM.

Angiogenesis, the growth of new vessels, is a complex process involving both the proliferation and migration of endothelial cells (EC). Myocardial ischemia is an especially potent inducer of angiogenesis in the heart. A variety of growth factors have been shown to induce angiogenesis in experimental models as determined with in vitro assays. One of these, the vascular endothelial growth factor (VEGF), functions through mechanisms involving the stimulation of EC growth (10, 12). These results imply that angiogenesis may occur in the DCM hamster, in which the main cause of cardiac dysfunction is microvascular disorder. However, the relationship between angiogenesis and the DCM hamster is not known. Moreover, the role of those growth factors in inducing angiogenesis has not been elucidated in this hamster.

Furthermore, it is demonstrated that in the development of heart failure, the extracellular matrix, its fibrillar collagen and myocyte hypertrophy could participate in cardiac remodeling. Coronary microvasculature is strongly involved in cardiac remodeling. Because MCI-154 exerts a positive inotropic action without increasing myocardial oxygen and energy consumption, it might beneficially affect cardiac remodeling, especially microvasculature. Moreover, amlodipine, which was revealed to reduce cardiac remodeling in the DCM hamster (26), might exert beneficial effects on the coronary microcirculation in this hamster. However, the relationship between angiogenesis and cardiac...
microvascular remodeling and the effects of such drugs on angiogenesis have not been elucidated in DCM.

The aim of the present study was to examine the long-term effects of amlodipine and MCI-154 on cardiac function and remodeling and to determine the effects of such drugs on myocardial microvascular remodeling in DCM.

**MATERIALS AND METHODS**

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, Revised 1985).

Experimental animals. All experiments were carried out using Bio 53.58 cardiomyopathic hamsters (Bio Breeders, Fitchburg, MA). At the age of 5 wk, 30 male Bio 53.58 hamsters were randomly assigned to receive either amlodipine (BIO-AM: 10 mg·kg$^{-1}$·day$^{-1}$ po; Pfizer Pharmaceutical), MCI-154 (BIO-M: 5 mg·kg$^{-1}$·day$^{-1}$ po; Mitsubishi Chemical, Tokyo, J apan), or standard chow (BIO-C) for 15 wk. Ten age-matched male F1b hamsters were used as controls. The dosage of 10 mg·kg$^{-1}$·day$^{-1}$ of amlodipine produced a substantial suppression of cardiac remodeling in rats with myocardial infarction (21) and DCM hamsters (26) without obvious hypotension. Because there has been no report about the effect of long-term MCI-154 treatment in the rodent model, the dosage of 5 mg·kg$^{-1}$·day$^{-1}$ was used in this study based on the results of preliminary tests.

Echocardiography. The method used for echocardiography was described previously (26). Briefly, each hamster was anesthetized with intraperitoneal injection of urethane (0.5 mg/body mass) and α-chloralose (100 mg/kg). Each hamster was then intraperitoneally administered zatebradine (3 mg/kg, Zeneca Pharmaceutical), a specific bradycardiac agent that selectively slows the depolarization in the pacemaker cells of the sinusoidal node without altering left cardiac contractility, even in the failing heart (18). Transthoracic echocardiograms (Hitachi EUB 565A, Hitachi, J apan) were obtained with a 7.5-MHz sector scanner, which gives a good resolution in the assessment of the small animal heart. M-mode echocardiograms at chordae levels were recorded at a paper speed of 100 mm/s, and the LV diastolic dimension (LVDd) and LV systolic dimension (LVDs) were measured by the leading-edge method of the American Society for Echocardiography for at least three consecutive cardiac cycles, after which the percent fractional shortening (%FS) was calculated as the percent difference between LVDs and LVDd: %FS = 100·(LVDd – LVDs)/LVDd. After heart rate was decreased to <350 beats/min by zatebradine, a pulsed-wave Doppler cursor with the transducer at the cardiac apex was placed in the area of the anterior mitral valve leaflet to capture the LV outflow tract envelope and the mitral inflow profile. The sample volume was placed near the tips of the mitral leaflets and adjusted to the position at which velocity was maximal and the flow pattern laminar. All Doppler spectra were recorded on paper at 100 mm/s and off-line as previously. Then early filling velocity (E wave), atrial filling velocity (A wave), and isovolumic relaxation time (IRT), defined as the interval from the valve artifactual end of the LV outflow tract until the beginning of transmitral inflow (E-wave), were measured.

Hemodynamic study. Animals were anesthetized with urethane and α-chloralose as in the echocardiography study and then artificially ventilated with oxygen-enriched air supplied by a Harvard respiratory system (tidal volume 1.2 ml, respiration rate 100 cycles/min). After a thoracotomy was performed with care taken to minimize the volume of bleeding, a 2-Fr microtip catheter manometer (SPC-320, Millar Instruments, Houston, TX) with a TCB-500 control unit (Millar Instruments) was inserted through the LV apex using a 22-gauge needle for puncture. As indexes of hemodynamics, the maximum rate of rise of ventricular pressure (dP/dt$_{max}$), the peak rate of pressure fall of ventricular pressure (dP/dt$_{max}$), the rate of the maximum velocity of shortening of unloaded contractile elements (V$_{max}$), and the time constant of the exponential fit of the time course of isovolumic pressure decline (tau) were obtained from LV pressure by analysis with a computer system (MP-100WS, BIOPAC System, Santa Barbara, CA) and the AcqKnowledge 2.0 program for the Macintosh (BIOPAC System).

Morphometric analysis. Hearts were removed, dipped into OCT compound (Tissue-Tek, Sakura Finetechinal, Tokyo, J apan), and then frozen in liquid nitrogen and stored at −80°C. Sections 16-μm thick were obtained from cross sections taken at the widest part of the LV. Double staining of sections was carried out to discriminate arteriolar and venular capillaries. Arteriolar capillaries were stained blue because they contained alkaline phosphatase, and venular capillaries were stained red because they contained dipeptidyl peptidase IV. Intermediate capillaries were stained violet because they contained both enzymes. Capillaries and myocytes were drawn using a projection tube attached to a microscope. The total numbers of capillaries and venular capillaries were counted, and the ratio of venular capillaries to total capillaries, the proportion of venular capillaries, was calculated.

Northern blot analysis. The cDNA probes used in the present study were cDNA for rat VEGF mRNA (5), sized ~600 bp, kindly provided by Dr. A. Asano (Hokkaido University, J apan) and cDNA for a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (no. 57091, American Type Culture Collection, Rockville, MD) for internal control. All cDNA probes were uniformly labeled with random primers using Klenow enzyme (Boehringer Mannheim) and α-[32P]dCTP (Life Science Products). Each preparation of total RNA was isolated from a LV tissue sample using TRIzol reagent (GIBCO BRL). Twenty micrograms of denatured RNA were size fractioned on 2% formaldehyde 1.2–1.5% agarose gels and then transferred onto a nylon membrane (Hybond-N, Amershams Life Science) overnight using 20× saline sodium citrate transfer buffer. Northern blot analysis was carried out according to the established method. Each membrane was exposed at −80°C on X-ray films (X-OMAT, Eastman Kodak) with a single intensifying screen for increasing exposure times to obtain signals in the linear range for densitometric analysis of each mRNA species. The GAPDH mRNA diffuse density score used as an internal control has been shown to be unchanged in the DCM hamster heart. To evaluate mRNA levels, an optical scanner (GT-9500, Seiko, Tokyo, J apan) was used to digitize autoradiograms. The density of autoradiogram bands in the digitized image was measured with the use of the public domain NIH image program and a computer (Macintosh Performa 6310, Apple Computer).

Statistical analysis. Results are expressed as means ± SD. Statistical analysis was performed by ANOVA with multiple comparisons by Fisher’s protected least significance t-test using StatView (Abacus Concept, Berkeley, CA).

**RESULTS**

Table 1 shows that there were no significant differences in the ratio of ventricular weight to body weight among the four groups.

In echocardiogram and hemodynamic studies, many parameters were used because the methods for cardiac
function have not been strictly established in small rodent models.

The results of echocardiography are shown in Table 2. In the M-mode echocardiogram, LVDd and LVDs of BIO-A and BIO-M were significantly decreased (P < 0.01, P < 0.01, respectively), and their %FS was significantly increased (P < 0.01, P < 0.01, respectively) compared with BIO-C. Therefore, LV systolic function was improved in BIO-A and BIO-M. Patterns of LV filling as recorded by diastolic Doppler mitral flow velocity were assessed to evaluate LV diastolic function. The E wave was not significantly different among any of the four groups, but the A wave was significantly increased in F1b compared with the other three DCM hamster groups, resulting in an increase in the ratio of LV filling as recorded by diastolic Doppler mitral flow max. The A wave, atrial filling velocity; IRT, isovolumic relaxation time. \(^*\)P < 0.01; \(^{\ddagger}\)P < 0.05 vs. BIO-C; \(^{\dagger}\)P < 0.01; \(^{\ddagger}\)P < 0.05 vs. BIO-A; \(^{\dagger}\)P < 0.01; \(^{\ddagger}\)P < 0.05 vs. BIO-M.

The results of the hemodynamic study are shown in Table 1. There were no significant differences in heart rate and peak LV pressure (LVP) among the four groups except that LVP in BIO-M was significantly decreased compared with the other three groups. LV end-diastolic pressures (LVEDP) of BIO-A and BIO-M were significantly smaller than those of BIO-C (P < 0.05, P < 0.05, respectively). The dP/dt\(_{\text{max}}\), which is highly sensitive to changes of contractility, was significantly increased in F1b compared with the other three DCM hamster groups, but there were no significant differences among the three DCM hamster groups. The results of the dP/dt\(_{\text{min}}\) were similar to those of dP/dt\(_{\text{max}}\). The V\(_{\text{max}, 5^{-1}}\), which is proposed as a measure of myocardial contractility that is independent of preload and afterload, was increased in BIO-A and BIO-M compared with BIO-C (P < 0.05, P < 0.05, respectively). Moreover, tau, which is an index of ventricular relaxation, was decreased in BIO-A and BIO-M compared with BIO-C (BIO-A: P < 0.05, BIO-M: not significant).

The morphometric analysis is shown in Fig. 1. Amlodipine and MCI-154 significantly increased the total capillary density (1,842 ± 168/mm\(^2\), 1,853 ± 288/mm\(^2\), respectively) compared with that of BIO-C (1,268 ± 183/mm\(^2\)), and that of F1b was significantly higher (1,963 ± 259/mm\(^2\)) than that of BIO-C. Moreover, amlodipine and MCI-154 significantly increased the proportion of venular capillaries, the ratio of venular to total capillaries (12.1 ± 4.6%, 8.6 ± 3.3%, respectively), compared with BIO-C (3.1 ± 2.1%), and that of F1b was significantly higher (21.6 ± 2.9%) than that of F1b.

Representative autoradiographs from the Northern blot analysis are shown in Fig. 2A. The level of mRNA expression for VEGF was highest in BIO-A and BIO-M. Figure 2B shows the relative ratios of mRNA expression of VEGF to GAPDH obtained from densitometric scanning of the four blots. Amlodipine and MCI-154 significantly increased the expression of mRNA for VEGF (130.1 ± 12.3%, 130.1 ± 11.7%, respectively) compared with BIO-C.

DISCUSSION

Effect of long-term treatment with amlodipine and MCI-154 on cardiac function and remodeling of DCM hamster heart. The hereditary DCM hamsters used in

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**Table 1. Effects of amlodipine and MCI-154 on ventricular weights and hemodynamic parameters in Bio 53.58 and F1b hamsters**

<table>
<thead>
<tr>
<th></th>
<th>BIO-C</th>
<th>BIO-A</th>
<th>BIO-M</th>
<th>F1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>113 ± 7</td>
<td>110 ± 4</td>
<td>105 ± 1</td>
<td>145 ± 8(^{\ddagger})</td>
</tr>
<tr>
<td>VV, mg</td>
<td>324 ± 21</td>
<td>347 ± 42</td>
<td>300 ± 13</td>
<td>437 ± 41(^{\ddagger})(^{\ddagger})</td>
</tr>
<tr>
<td>VV/BW, mg/g</td>
<td>2.9 ± 0.1</td>
<td>3.2 ± 0.4</td>
<td>2.9 ± 0.1</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>435 ± 31</td>
<td>430 ± 26</td>
<td>432 ± 21</td>
<td>428 ± 15</td>
</tr>
<tr>
<td>LVFP, mmHg</td>
<td>82 ± 16</td>
<td>76 ± 26</td>
<td>46 ± 17(^{\ddagger})</td>
<td>87 ± 11(^{\dagger})</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>14 ± 7</td>
<td>5 ± 3(^{\ddagger})</td>
<td>5 ± 3(^{\ddagger})</td>
<td>5 ± 2(^{\ddagger})</td>
</tr>
<tr>
<td>dP/dt(_{\text{max}}), mmHg/s</td>
<td>5,820 ± 1,476</td>
<td>5,577 ± 280</td>
<td>5,682 ± 421</td>
<td>11,289 ± 2,735(^{\ddagger})</td>
</tr>
<tr>
<td>dP/dt(_{\text{min}}), mmHg/s</td>
<td>3,974 ± 406</td>
<td>4,424 ± 2,350</td>
<td>4,788 ± 564</td>
<td>6,873 ± 1,156(^{\ddagger})</td>
</tr>
<tr>
<td>V(_{\text{max}, 5^{-1}})</td>
<td>264 ± 12</td>
<td>294 ± 40(^{\ddagger})</td>
<td>297 ± 15(^{\ddagger})</td>
<td>683 ± 318(^{\ddagger})</td>
</tr>
<tr>
<td>Tau</td>
<td>9.4 ± 4.6</td>
<td>6.7 ± 1.5(^{\ddagger})</td>
<td>8.8 ± 1.9</td>
<td>7.0 ± 3.3(^{\ddagger})</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10 hamsters. LVDd, LV diastolic dimension; LVDs, LV systolic dimension; %FS, percent fractional shortening; E wave, early filling velocity; A wave, atrial filling velocity; IRT, isovolumic relaxation time. \(^*\)P < 0.01; \(^{\ddagger}\)P < 0.05 vs. BIO-C; \(^{\dagger}\)P < 0.01; \(^{\ddagger}\)P < 0.05 vs. BIO-A; \(^{\dagger}\)P < 0.01 vs. BIO-M.

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**Table 2. Echocardiographic measurements in Bio 53.58 and F1b hamsters**

<table>
<thead>
<tr>
<th></th>
<th>BIO-C</th>
<th>BIO-A</th>
<th>BIO-M</th>
<th>F1b</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDd, mm</td>
<td>6.9 ± 0.4(^{*})</td>
<td>5.8 ± 0.4(^{*})</td>
<td>5.8 ± 0.3(^{*})</td>
<td>4.5 ± 0.2(^{*})</td>
</tr>
<tr>
<td>LVDs, mm</td>
<td>5.3 ± 0.4</td>
<td>4.0 ± 0.3(^{*})</td>
<td>3.9 ± 0.3(^{*})</td>
<td>1.7 ± 0.1(^{*})</td>
</tr>
<tr>
<td>%FS, %</td>
<td>23 ± 3</td>
<td>32 ± 2(^{\ddagger})</td>
<td>32 ± 3(^{\ddagger})</td>
<td>62 ± 2(^{\ddagger})</td>
</tr>
<tr>
<td>E wave, cm/s</td>
<td>82 ± 11</td>
<td>78 ± 4</td>
<td>76 ± 9</td>
<td>86 ± 9</td>
</tr>
<tr>
<td>A wave, cm/s</td>
<td>46 ± 8</td>
<td>50 ± 3</td>
<td>46 ± 10</td>
<td>66 ± 6(^{\ddagger})</td>
</tr>
<tr>
<td>E/A</td>
<td>1.8 ± 0.3</td>
<td>1.6 ± 0.1(^{\ddagger})</td>
<td>1.7 ± 0.2</td>
<td>1.3 ± 0.1(^{\ddagger})(^{\ddagger})</td>
</tr>
<tr>
<td>IRT, ms</td>
<td>29.2 ± 4.5</td>
<td>23.6 ± 1.1(^{\dagger})</td>
<td>21.3 ± 2.1(^{\dagger})</td>
<td>18.2 ± 2.5(^{\dagger})</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10 hamsters. LVDd, LV diastolic dimension; LVDs, LV systolic dimension; %FS, percent fractional shortening; E wave, early filling velocity; A wave, atrial filling velocity; IRT, isovolumic relaxation time. \(^*\)P < 0.01; \(^{\ddagger}\)P < 0.05 vs. BIO-C; \(^{\dagger}\)P < 0.01; \(^{\ddagger}\)P < 0.05 vs. BIO-A; \(^{\dagger}\)P < 0.01 vs. BIO-M.
this study have been used as an animal model for human cardiomyopathy and heart failure. It is well established that the disease process affects myocardial tissue greatly in inhomogeneous ways as evidenced by focal cell loss, microvascular spasms (6), inhomogeneous capillary flow (7) and resultant focal ischemic areas, and marked heterogeneity in cellular calcium content (28). Recently, a defect in the gene for \( \delta \)-sarcoglycan, dystrophin-associated glycoprotein, was reported in the cardiomyopathic hamster (19). Many studies have reported the cardioproteective effects of different drugs, especially calcium channel antagonists (6) and angiotensin-converting enzyme inhibitors (9). Calcium channel antagonists are theoretically effective in the prevention of LV remodeling because of their actions as arteriolar dilators and anti-ischemic agents. We previously reported that amlodipine inhibits the deterioration of LV function and reduces the increase of fibrotic tissues and the decrease of the numbers of cardiomyocytes in DCM hamsters (26). The mechanism by which amlodipine acts on the DCM hamster is not clear, but we showed that it acts without alteration of the calcium handling of the LV (26). Recent studies suggest that amlodipine releases nitric oxide from blood vessels in vitro (30) and has cardioprotective effects owing to suppression of the production of inducible nitric oxide synthase in a mouse model of myocarditis (25) and effects that other calcium channel antagonists such as verapamil, diltiazem, and nifedipine do not have. In our preliminary study (data not shown), nifedipine did not exert beneficial effects on cardiac performance in DCM hamsters. Thus amlodipine might have unique effects on the failing heart that other calcium antagonists do not have, aside from having fewer negative inotropic effects than other calcium channel antagonists and not activating the neurohormonal system due to its slow onset of action and plasma half-life of more than 30 h.

In this study, MCI-154, as well as amlodipine, improved cardiac systolic and diastolic functions, and to our knowledge, we are the first to show the beneficial cardioprotective effect of long-term treatment with MCI-154. Heart failure is associated with pathobiological changes that can reduce the responsiveness of the myocardiium to positive inotropic agents. However, positive inotropic compounds, phosphodiesterase inhibitors, are harmful in the long-term treatment of chronic congestive heart failure (15), because they may induce a calcium overload, unwanted changes in cross-bridge kinetics, and an acceleration in heart rate. The energy cost for the newly developed Ca\(^{2+}\) sensitizer is lower than that for catecholamine and phosphodiesterase inhibitors (22). Ca\(^{2+}\) sensizers may be able to generate force with smaller amounts of Ca\(^{2+}\) by increasing the responsiveness of myofilaments to Ca\(^{2+}\), potentially reducing myocardial oxygen consumption (8). It has been demonstrated that MCI-154 has a vasodilating effect, predominantly on veins (14), and preload reduction caused by this venodilating effect may have decreased LV wall stress and hence had an oxygen-saving effect. Additionally, the Ca\(^{2+}\)-sensitizing effect of MCI-154 might have reduced the energy requirement for Ca\(^{2+}\) sequestration by sarcoplasmic reticulum, thereby overcoming the oxygen-wasting effect of its positive inotropic action, and thus reducing oxygen consumption.

Structural changes of the myocardium, referred to as remodeling, have profound effects on the performance of the LV and on long-term prognosis. In this study, amlodipine and MCI-154 inhibited the progression of the impairment of LV contractility, which was demonstrated by the %FS, and it prevented LV dilatation, so it inhibited ventricular remodeling. Furthermore, both amlodipine and MCI-154 improved not only systolic function but also diastolic function, as indicated by the IRT obtained from echocardiography. IRT determination by pulse Doppler echocardiography is noninvasive and closely correlates with the diastolic time constant (20). Amlodipine significantly decreased the diastolic time constant in the hemodynamic study, and this coincided with the data of the echocardiography study. In addition, E/A, examined by transmitral flow analysis using pulse Doppler echocardiography, was signifi-
significantly decreased in amlodipine-treated hamsters compared with the nontreated group, and the E/A of the normal F1b hamster was significantly lower than that of the cardiomyopathic hamster groups. This indicated that the LV diastolic function of cardiomyopathic hamsters without treatment appeared to have a pseudonormalized pattern, with increased peak E wave velocity, decreased peak A wave velocity, and rapid E wave deceleration. Amlodipine (significantly) and MCI-154 (but not significantly) improved these parameters. In a rat coronary ligation model, amlodipine improved the pseudonormalized pattern, as assessed by transmittal flow using pulse Doppler echocardiography, in myocardial infarction (21). Therefore, it is considered that amlodipine and MCI-154 suppressed the progression of stiffness caused by advancing fibrosis in the DCM hamster.

Abnormality of microvasculature and mechanism by which amlodipine and MCI-154 induced angiogenesis in the DCM hamster heart. In the present study, not only the total number of capillaries but also the proportion of venular capillaries were significantly increased in the DCM hamsters treated with amlodipine and MCI-154. Because capillary angiogenesis usually initiates from the venular site, an increase in the proportion of venular capillaries indicates that much more promotion of angiogenesis occurs (2). The total number of capillaries and the proportion of venular capillaries were actually increased in the risk area of ischemic myocardium in a rat coronary ligation model (29). Furthermore, this study demonstrated by Northern blot analysis for VEGF that more growth factor-inducible angiogenesis occurred in the LV of the DCM hamsters treated with amlodipine and MCI-154 than in untreated hamsters. VEGF is a potent growth factor inducing angiogenesis, and it is strongly expressed in the ischemic myocardium (10, 12). Because the myocardium in untreated DCM hamsters was much more injured and at risk of ischemia than in hamsters treated with amlodipine and MCI-154, which inhibited remodeling of the LV myocardium, growth factors such as VEGF should be more highly expressed in untreated animals. However, this study showed that their expression was greater after treatment with amlodipine and MCI-154. Several possible explanations for this exist. VEGF is mainly produced by myocytes in the LV (4, 12), and cardiac myocytes overexpress VEGF in ischemic myocardium (11). Thus, because of the cardioprotective effects of amlodipine and MCI-154, more VEGF might be produced in the DCM hamsters treated with amlodipine and MCI-154.

Moreover, as shown above, treatment with amlodipine and MCI-154 for 15 wk improved both systolic and diastolic functions. The relationship between cardiac capillarization and cardiac function is not fully elucidated. Because it has been suggested that changes in the vessel wall geometry and, consequently, wall tension might induce growth of new vessels (27), it is possible that higher wall stress, connected with increased blood flow induced by the improved diastolic function of the LV, might produce slight damage to the capillary endothelium, which would lead to the release of protease, degradation of the basement membrane, and subsequent endothelial migration and mitosis. Furthermore, Sladek et al. (22) reported a dose correlation between LVEDP and capillary density in the cardiac tissue close to the infarcted zone in a rat model of coronary ligation. Accordingly, the improve-

![Image](https://example.com/image.png)

**Fig. 2.** Northern blot analysis demonstrating expression of vascular endothelial growth factor (VEGF) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in untreated Bio 53.58 hamsters (BIO-C) and those treated with amlodipine (BIO-A) and MCI-154 (BIO-M). F1b rats were used as controls. A: representative Northern blot analysis of VEGF and GAPDH mRNA. Expression of VEGF mRNA is significantly higher in BIO-A and BIO-M than in BIO-C. B: bar graph showing abundance of VEGF mRNA relative to GAPDH mRNA as corrected for respective time controls (100%). Level of VEGF mRNA is significantly increased by 30.1% in BIO-A and BIO-M compared with BIO-C. N = no. of hamsters. *P < 0.05 vs. BIO-C.
ment of cardiac performance caused by amlodipine and MCI-154 may induce angiogenesis in the LV of the DCM hamsters.

Study limitations. We examined only the expression of mRNA of VEGF and did not assess the amount and localization of the expression of VEGF protein and the function of its receptors flt-1 and flk-1, which VEGF binds to and which are expressed on EC. Several cytokines, including transforming growth factors-α and -β, tumor necrosis factor-α, interleukin-1β, stimulation of protein kinase C activity and stretching of the myocardium, increase VEGF expression, but we have not assessed the relationship between the expression of VEGF and those effectors in the DCM hamster. Moreover, the functions of other direct-acting endothelial factors, i.e., acidic and basic fibroblast growth factor, platelet-derived growth factor, and angiopoietin 1, etc., should be clarified to understand the functional roles of coronary microvascular remodeling in DCM.

In conclusion, the present study examined the long-term effects of amlodipine and MCI-154 on cardiac performance, coronary capillaries, and induction of angiogenesis in DCM hamsters. Treatment with amlodipine and MCI-154 for 15 wk improved both systolic and diastolic functions expressed by %FS and IRT, which were reduced in the DCM hamster heart. VEGF was increased in LV tissues treated with amlodipine and MCI-154, though it was suppressed in the DCM hamster heart. Long-term treatment with amlodipine and MCI-154 may induce angiogenesis in DCM, and this effect may lead to improved cardiac performance.

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