Heart failure and greater infarct expansion in middle-aged mice: a relevant model for postinfarction failure

KENNETH E. GOULD,1 GEORGE E. TAFFET,2 LLOYD H. MICHAEL,2 ROBERT M. CHRISTIE,1 DEBRA L. KONKOL,1 JENNIFER S. POCIUS,2 JUSTIN P. ZACHARIAH,2 DAMIAN F. CHAUPIN,2 SHERITA L. DANIEL,2 GEORGE E. SANDUSKY, JR.,1 CRAIG J. HARTLEY,2 AND MARK L. ENTMAN2

1Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285; and 2DeBakey Heart Center and Department of Medicine, Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77030

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Gould, Kenneth E., George E. Taffet, Lloyd H. Michael, Robert M. Christie, Debra L. Konkol, Jennifer S. Pocius, Justin P. Zachariah, Damian F. Chaupin, Sherita L. Daniel, George E. Sandusky, Jr., Craig J. Hartley, and Mark L. Entman. Heart failure and greater infarct expansion in middle-aged mice: a relevant model for postinfarction failure. Am J Physiol Heart Circ Physiol 282: H615–H621, 2002.—Young mice tolerate myocardial loss after coronary artery ligation (CAL) without congestive heart failure (CHF) signs or mortality. We predicted a CHF phenotype after CAL in aged mice. Left coronary artery ligation produced permanent myocardial infarcts (MI). Mortality was higher in male 14-mo-old C57BL/6N mice (Older mice) than in 2-mo-old mice (Young mice) (16 of 25 Older mice died vs. 0 of 10 Young mice, P < 0.02). After 8 wk, rales, weight loss, and lethargy preceded death. Captopril (50 mg·kg−1·day−1) increased Older mouse survival (6 of 22 died, P < 0.02). Captopril improved systolic function (peak aortic blood velocity) from 76 ± 6% of baseline in untreated Older mice to 93 ± 8% (P < 0.036). At 24 h, MI comprised 28 ± 4% of the left ventricle in Young mice, surprisingly larger than that in Older mice (18 ± 2%, P < 0.011). Endocardial area underlying the infarct scar was significantly larger in Older mice than in Young mice. Captopril did not reduce expansion but markedly reduced septal hypertrophy. Aging reduces compensatory ability in mice despite smaller acute infarcts. Less effective myocardial repair, greater infarct expansion, and septal hypertrophy are seen with aging. Aging is a more relevant murine model of post-MI heart failure in patients.

Heart failure; myocardial infarction; aging; mouse

CONGESTIVE HEART FAILURE (CHF) is an increasingly frequent cause of cardiovascular morbidity and mortality, with ~400,000 new cases reported annually (14, 20). Survival 5 yr after the diagnosis of CHF is poor, ranging as low as 25–35% (8). The loss of functional left ventricular (LV) myocardium after myocardial infarction has replaced hypertension as the primary cause for CHF due to systolic dysfunction (5).

In contrast to the relatively high propensity to develop CHF after myocardial infarction in the human, this syndrome has been difficult to demonstrate in the mouse. In our earlier work and that of others (7, 12, 15, 16, 18), despite clear evidence of depressed systolic and diastolic cardiac function, there was no evidence of a CHF syndrome in young (2–3 mo) mice after coronary artery ligation (CAL). In our hands, mice lived more than 12 mo after CAL with persistently decreased LV systolic function without excess mortality or signs of CHF. To study the biology of heart failure in genetically altered mice, the most clinically relevant etiology was myocardial infarction. Such a model would allow us to reproduce principal aspects of cardiac pathology associated with coronary artery disease and subsequent heart failure, including ischemia, inflammation (3), fibrosis associated with healing of the infarct, asymmetrical loading of the surviving LV and infarct scar (13, 25), compensatory hypertrophy, and ventricular remodeling (15).

The hypothesis of this study was that the use of young mice might have altered the response to myocardial infarction and that older mice might respond in a manner similar to the population in which clinical infarction occurs. Our data show increased mortality, an increase in observable signs of late-stage heart failure, and greater infarct expansion in mice that were at the middle of their life span (14 mo old) at the time of infarction produced by CAL compared with typical 2-mo-old mice. Treatment with the angiotensin-converting enzyme inhibitor captopril increased survival and improved hemodynamic function of older infarcted mice, a finding typical of human CHF. However, captopril did not influence infarct expansion but markedly reduced septal hypertrophy.

METHODS

Male C57BL/6N mice (Harlan Sprague Dawley; Indianapolis, IN, and Houston, TX) from three age groups were used:
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2 mo old (Young mice), 10–12 mo old, and 14 mo old (Older mice). Use of mice was approved by the Institutional Animal Care and Use Committees of the American Association for Accreditation of Laboratory Animal Care-accredited institutions, Lilly Research Laboratories, and Baylor College of Medicine in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Revised 1996).

Permanent myocardial infarcts were produced by ligation of the anterior descending branch of the left coronary artery (15, 16). Briefly, mice were anesthetized with intraperitoneal pentobarbital sodium (4 mg/ml, 14 µl/g body wt, Nembutal, Abbott Laboratories; North Chicago, IL), placed in a supine position, and intubated. Mice were ventilated with a mixture of 100% oxygen and room air with a volume-cycled rodent ventilator (100 cycles/min, Harvard Apparatus; Holliston, MA). Ventilator stroke volume was adjusted to fully inflate but not overexpand the lungs. Each paw was taped to a copper electrode for bipolar lead electrocardiographic recordings. A median sternotomy was performed, exposing the anterior surface of the heart. The anterior descending branch of the left main coronary artery (LAD) was ligated at a position ~1 mm from the tip of the normally positioned left auricle with a 8-0 polypropylene monofilament suture (Prolene, Ethicon; Somerville, NJ). The ligature was not removed after placement. Sham operations were created by passing the suture under the coronary artery at the position used for ligation and leaving a short piece of suture in place underneath the artery without ever constricting the artery. After chest closure, mice were recovered in a supine position, warmed, and provided with 100% oxygen by nose cone. Analgesia was extended with subcutaneous 2.5 mg/kg buprenorphine HCl (Buprenex, Reckitt and Colman Pharmaceuticals; Richmond, VA). Mice recovered overnight in warmed housing with a nebulized oxygen atmosphere and must have survived 24 h after surgery to be included in the study. Mice assigned to control groups did not receive any surgical treatment. All mice were observed daily.

captopril (Sigma; St. Louis, MO) was dissolved in water and administered by gavage twice daily at 25 mg/kg at each administration, for a total daily dose of 50 mg/kg. The gavage volume was 100 µl. To equalize handling, all mice not receiving captopril were gavaged with an equal amount of water on the same schedule.

Infarct size and the area of myocardium at risk of infarction were measured using 1% Evans blue dye and 1.0% 2,3,5-triphenyltetrazolium chloride (TTC), followed by image analysis as previously reported (15, 16).

Cardiac function was evaluated noninvasively with high-frequency pulsed Doppler ultrasound (6, 7, 21). Mice were anesthetized with a mixture of xylazine (1.4 mg/ml, Xylazine-20, Butler; Columbus, OH), ketamine HCl (42.8 mg/ml, Ketaset, Fort Dodge; Fort Dodge, IA), and acepromazine maleate (1.4 mg/ml, Butler; Columbus, OH) administered intraperitoneally at 0.5 µl of the mixture/g body wt. With mice supine, a 2.0-mm-diameter 10-MHz transducer, mounted at the tip of a stainless steel probe, was placed at the xiphoid process with light pressure. Body fur at the left lower sternal border was clipped lightly, and the skin was wetted with water to improve sound transmission. Optimal Doppler audio flow signals from the outflow tract (aortic root) were obtained by adjusting the position of the transducer on the chest surface and by setting the pulsed Doppler range gate between 4 and 7 mm. The R wave of the electrocardiogram was used as a timing signal and was superimposed on the Doppler spectral display. Multiple sets of signals were obtained from each mouse to allow for variation in heart rate and to assess the consistency of the signals. Doppler signals were acquired for a minimum of 10 s and a maximum of 30 s from the aortic root, yielding up to 150 cardiac cycles for analysis. In the majority of mice, 15–25 beats were analyzed. The pulsed Doppler probes, amplifiers, and range gate circuits were custom made in our own laboratories (6, 7).

An analog spectrum analyzer (Vasculab SP-25A, Medasonics; Mountain View, CA) processed Doppler audio signals for direct viewing and for operator feedback in positioning the probe. Audio signals were simultaneously acquired at 64 kHz and digitized for analysis on a desktop computer programmed and optimized for use with mice (LabVIEW version 4.0, National Instruments; Austin, TX, and VI Engineering; Indianapolis, IN). Each of the treatment groups was followed longitudinally with ultrasound over the time course of the experiment. Data are expressed as a percentage of the individual mouse preprocedure values.

To assess changes in cardiac structure, serial cross sections of the heart were evaluated planimetrically (15). As previously reported (15), hearts were obtained in diastole after cardioplegia was achieved by jugular vein infusion of the following solution (in g/l): 4.0 NaCl, 3.7 KCl, 1.0 NaHCO3, 2.0 glucose, 3.0 2,3-butanedione monoxime, 3.8 EGTA, and 0.0002 nifedipine (Sigma). Solution pH was adjusted to 7.4. After cardiac standstill was obtained, hearts were excised and rinsed in cold cardioplegia solution. Hearts were fixed for 10 min by retrograde aortic perfusion of 10% neutral buffered zinc-formalin (Z-fix, Anatech; Battle Creek, MI) while immersed in the same fixative. Constant intraventricular fixa- tion pressure of ~16 cmH2O was maintained by holding the LV drain 16 cm above the base of the heart. Hearts were fixed by further immersion, embedded in paraffin, and sectioned for hematoxylin and eosin or Masson’s trichrome staining.

Mass of the LV and interventricular septum and the ventricular expansion ratio (VER) were obtained as previously reported (15).

Statistical analysis of all data was carried out using the SAS and JMP analysis platforms (SAS version 6.12 and JMP version 3.2.2, SAS Institute; Cary, NC). Survival was calculated by a log-rank test for homogeneity. Hemodynamic data from pulsed Doppler ultrasound examinations were analyzed by a mixed-effects repeated-measures ANOVA. The model accounted for effects of treatment group, time of evaluation, interaction of treatment and time, and heterogeneous variance caused by deaths before the end of a study. The appropriate treatment-by-time interaction contrast and least-squares means were used to assess the magnitude of treatment differences. Anatomic data were analyzed by one-way ANOVA with a contrasting Tukey-Kaplan honest significant differences test or Student’s t-test corrected for multiple comparisons as appropriate. Multivariate comparison of interventricular septal mass data and VER was made with a two-sample Hotelling T2-test. All data are reported as means ± SE unless indicated. For all tests, P < 0.05 was used to identify significant differences.

RESULTS

Infarct size 24 h after ligation. Consistent with our prior experience, ligation of the LAD in 2-mo-old C57BL/6 mice (Young mice) placed 43 ± 4% (Fig. 1) of the LV area at risk (AAR) and produced infarctions of 28 ± 4% of the LV when assessed 24 h after CAL (16). Almost 70% (67 ± 4%) of the AAR after CAL could not metabolize the TTC stain at 24 h in Young mice and therefore was considered infarcted. When the LAD was
occluded at this location in Older mice, there were no age differences in the LV AAR. Surprisingly, the resulting myocardial infarcts were only 18 ± 2% of the LV, significantly smaller than in the Young mice (P < 0.01 vs. Young mice; Fig. 1). The infarction in Older mice represented only 41 ± 4% of the AAR, significantly less than that observed in Young mice at 24 h (P < 0.001; Fig. 1).

Survival after myocardial infarction. We examined survival in Older mice after CAL (Fig. 2A) compared with young mice. Older mice experienced mortality that was substantially greater than the Young mice (P < 0.001 vs. Older mice). Only 9 of 25 (36%) Older mice survived to the end of the observation period, 56 days after infarction, in contrast to 10 of 10 Young mice (P < 0.02). Ninety-two percent of the sham-operated Older mice survived 8 wk (P < 0.002 vs. untreated infarcted mice; Fig. 2B).

We then examined mice aged 10–12 mo old (Fig. 2A). Mice 10–12 mo old experienced mortality higher than Young mice (P < 0.006 vs. 2-mo-old mice) but not as great as in Older mice (P < 0.05 vs. 14-mo-old mice). There were no obvious differences in the timing of deaths between the 10- to 12-mo-old and Older mouse groups.

Survival of Older mice was modified after permanent CAL by treatment with the angiotensin-converting enzyme inhibitor captopril (Fig. 2B). Captopril was begun on the morning of the eighth postoperative day (25 mg/kg twice each day) and produced a significant increase in survival in the 14-mo-old mice after infarction. In this group, 16 of 22 (73%) mice survived 8 wk (P < 0.016 vs. untreated Older mouse group).

Clinical characteristics of mice with heart failure. Over 50% of Older mice developed characteristics after CAL that were different from Young mice. These mice groomed poorly and became increasingly inactive. They showed respiratory distress and clearly visible use of accessory muscles to breathe. Orthostatic apnea was inducible when these mice were placed in dorsal recumbency and was relieved if mice were promptly returned to the sternal position. Rales were occasionally audible but not persistent in individual mice. These findings were also present in those captopril-treated mice that decompensated, as an antecedent to death.

Another part of the observed heart failure syndrome was weight loss. Untreated Older mice that died early (range: 3–46 days survived) weighed 77.6 ± 3.6% of their preocclusion weight shortly before dying (Table 1). Surviving untreated Older mice (all at 56 days) weighed 91.1 ± 1.4% of their preocclusion weight (P < 0.05 vs. early deaths). Young mice maintained or gained weight significantly better than Older mice in both the sham-operated (P < 0.001 vs. Older mice) and infarcted groups (P < 0.001 vs. Older mice) (Table 1).

Captopril treatment confounded the use of weight loss as a marker of decompensation, perhaps by causing weight loss in all groups. Captopril-treated mice that died early (range: 3–34 days) weighed 83.9 ± 5.8% of their starting weight, which was not different from captopril-treated mice that survived to 56 days [86.03 ± 1.6%; not significant (NS) vs. early treated deaths].

Fig. 1. Infarct size and area at risk after permanent left anterior descending coronary artery occlusion in 3- to 4-mo-old mice (n = 12; Young mice) and 14-mo-old mice (n = 13; Older mice). Hearts were removed at necropsy 24 h after occlusion. Data are means ± SE. *P < 0.01 vs. Older mice for infarct/left ventricle (LV); **P < 0.001 vs. Older mice for infarct/area at risk.

Fig. 2. A: first study of survival to 8 wk after myocardial infarction (MI) at different ages without drug treatment (Young mice: 10 of 10 survived to 8 wk; 10- to 12-mo-old mice: 11 of 18 survived, *P < 0.006 vs. Young mice; Older mice: 3 of 8 survived, *P < 0.047 vs. 10–12 mo old and ‡P < 0.0001 vs. Young mice). B: survival of Older mice to 8 wk after MI and captopril treatment (control: 9 of 9 survived; sham operated: 12 of 13 survived; infarcted captopril treated: 16 of 22 survived, not significant vs. sham-operated mice; infarcted untreated: 9 of 25 survived, *P < 0.02 vs. survival of infarcted Young mice, ‡P < 0.002 vs. sham-operated mice, †P < 0.016 vs. infarcted untreated mice).
Table 1. Body weight changes after MI and captopril treatment

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<th>Body Weight, g</th>
<th>%Body Weight</th>
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<tr>
<td><strong>Sham-operated mice</strong></td>
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<tr>
<td>Older</td>
<td>28</td>
<td>33.2 ± 0.9</td>
<td>89.75 ± 3.9</td>
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<td>Young</td>
<td>10</td>
<td>32.3 ± 0.95</td>
<td>115.8 ± 4.2‡</td>
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<tr>
<td><strong>MI survived</strong></td>
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</tr>
<tr>
<td>Older</td>
<td>14</td>
<td>32.6 ± 0.9</td>
<td>91.1 ± 1.4</td>
</tr>
<tr>
<td>Untreated</td>
<td>15</td>
<td>31.6 ± 0.7</td>
<td>86.03 ± 1.6</td>
</tr>
<tr>
<td>Captopril treated</td>
<td>10</td>
<td>28.3 ± 0.9</td>
<td>118.8 ± 1.8†</td>
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<td><strong>MI early death</strong></td>
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<tr>
<td>Older</td>
<td>8</td>
<td>26.5 ± 1.4</td>
<td>77.6 ± 3.6‡</td>
</tr>
<tr>
<td>Untreated</td>
<td>3</td>
<td>29 ± 1.2</td>
<td>83.9 ± 5.5</td>
</tr>
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Data are means ± SE; n = no. of mice. MI, myocardial infarction; Older mice, mice aged 14 mo; Young mice, mice aged 2 mo; %Body weight, percentage of body weight on day of coronary occlusion. Weights were obtained from sham-operated and survived mice 8 wk after surgery. Weights were obtained from early deaths on the day of death. *P < 0.05 vs. Older untreated survived mice; †P < 0.001 vs. Older survived mice with or without captopril; ‡P < 0.001 vs. Older sham-operated mice.

**Hemodynamics.** Serial studies of blood velocity measurements obtained at the aortic root with pulsed Doppler ultrasound showed progressively greater differences in systolic function when comparing 1) mice that survived to the end of the experiment versus those that died early and 2) untreated versus captopril-treated Older mouse groups (Fig. 3). Two weeks after coronary occlusion, peak aortic velocity in untreated Older mice that survived to the end of the experiment decreased to 85 ± 5% of the preocclusion baseline value. This was a larger decline than that observed in mice that had been receiving captopril for 7 days at the time of the 2-wk measurement (captopril-treated mice: 95 ± 4% of baseline). However, peak aortic velocity in untreated Older mice that died early decreased to 76 ± 2% of the preocclusion baseline value 2 wk after infarction (P < 0.0011 vs. captopril-treated mice). For all groups, mean heart rate was 300–325 beats/min at baseline and was not altered by age, procedure, drug treatment, or outcome.

One month after coronary occlusion, peak aortic velocity declined only slightly in mice receiving captopril (94 ± 6% of baseline) and was almost unchanged in untreated surviving mice compared with the values at 2 wk (84 ± 5% of baseline, NS vs. captopril-treated mice). Peak aortic velocity continued to fall sharply in the group of untreated mice that died early, reaching 70 ± 3% of the preinfarction value (P < 0.026 vs. untreated surviving mice, P < 0.0001 vs. captopril-treated mice). Subsequent mortality prevented obtaining Doppler ultrasound data at later time points from the group of decompensating mice.

Two months after coronary occlusion, peak aortic velocity was 93 ± 8% of the baseline value in captopril-treated mice. However, surviving untreated mice had declined to 76 ± 6% of the preinfarction baseline (P < 0.04 vs. untreated surviving mice). This value represented a continued decline in systolic function.

**Light microscopy of myocardial infaracts.** Chronic anterior transmural infarcts were observed in the LV of untreated mice that survived to 8 wk. Infaracts included the apex and a major part of the distal ventricular free wall, displaying aneurysmal formation with loss of most viable myocytes characteristic of both old and young mice (16). This appearance was unchanged by captopril in treated mice that survived to 8 wk.

As previously reported (15), we measured the total noninfarcted ventricular mass and individually measured the mass of the interventricular septum. We assessed ventricular dilatation by obtaining the ratio of the endocardial area underlying the infarct to the total endocardial area of the LV. This value, expressed as a percentage, was referred to as the VER. Ventricular dilatation, assessed by VER, was significantly greater after myocardial infarction in untreated Older mice than in young mice (26.5 ± 3.8% untreated Older mice vs. 15.7 ± 1.2% Young mice, P < 0.05; Fig. 4B). Total noninfarcted ventricular mass did not show a signifi-
cant increase after infarction in Older mice (sham: 53.48 ± 1.97 mg, n = 6; infarcted: 57.47 ± 6.2 mg, n = 6). However, the septum, which was not infarcted in any mouse after coronary occlusion, showed significantly greater mass in untreated Older mice (24.7 ± 4.2 mg, P < 0.02 vs. Older sham-operated and infarcted Young mice) than in sham-operated Older mice (14.97 ± 1.6 mg) or in infarcted Young mice (17.1 ± 1.4 mg) (Fig. 4A). Captopril treatment of infarcted Older mice reduced septal mass to the levels found in sham-operated mice (16.9 ± 2.6 mg in captopril-treated vs. 15 ± 1.2 mg in sham-operated mice) (Fig. 4A). The response observed in the septum was not reflected in ventricular chamber geometry, however, because the expansion ratio did not change in infarcted Older mice that received captopril (32.8 ± 5.2% in captopril-treated vs. 26.5 ± 3.8% in untreated mice, P = NS) (Fig. 4B).

Multivariate analysis of the septal mass and expansion ratios showed that infarcted mice were separated into two populations that differed significantly in their anatomic response to ischemic ventricular injury. The distinguishing characteristic of the populations was their age at the time of coronary occlusion (Fig. 4C). Captopril did not appear to reduce infarct expansion but markedly reduced septal hypertrophy in Older mice (Fig. 4D).

DISCUSSION

CHF is an increasingly common outcome of myocardial infarction in the United States today (5). In the present study, a heart failure syndrome and increased mortality were found after myocardial infarcts created by CAL in wild-type mice at the middle of their lifespan. This finding is in contrast to the outcome in mice infarcted when 2 mo old (Young mice), in which no heart failure, normal activity, preserved body weight, and very infrequent death were observed for as long as 12 mo after ligation (15). Young mice had persistent and significant cardiac systolic dysfunction as measured by ejection fraction and peak aortic flow veloci-
ties (7, 15). The frequent development of CHF in old mice is reminiscent of the higher likelihood of the development after myocardial infarction in aging humans (5, 10) and is substantially different from our experience with young adult mice, those typically used for cardiovascular studies. Additionally, increasing age is associated with increased mortality after myocardial infarction in many studies (1, 11, 22, 23). Importantly, these mice in our hands survive beyond 2 yr, so 14 mo reflects “late middle age,” not senescence.

We initially sought to create postinfarction CHF in young wild-type mice but were unable to do so. Male C57BL/6 mice aged 8–10 wk old at the time of coronary occlusion compensated for the substantial injury produced by occlusion of a large coronary artery. Mice of this age had significant decreases in peak aortic velocity and peak diastolic early filling velocity, as shown by pulsed Doppler ultrasound (15). Ejection fractions, determined by $^{178}$Ta ventriculography, were 26.4% at 2 mo after infarction, a 50% reduction from control values (7). Furthermore, infarcted young mice developed none of the clinical characteristics of heart failure even when challenged with 8% dietary salt in the presence of high doses of deoxycorticosterone or when allowed to survive more than 1 yr post-CAL (unpublished data). Therefore, we conclude that myocardial infarction in the young adult mouse is not an optimal model to study the prevention of CHF or accelerated mortality associated with heart failure because the young mouse tolerates extensive myocardial loss without experiencing these clinically relevant endpoints. In contrast, induction of myocardial infarction in the older mouse did result in these outcomes, and they experienced improved survival and reduced septal hypertrophy after angiotensin-converting enzyme inhibition. This latter model is obviously more relevant to the clinical syndrome.

We expected that the infarcts would be larger in the Older mice, consistent with the higher mortality. To our surprise, at 24 h, the infarcted LV mass was significantly smaller than that seen in Young animals.

In summary, the older male mouse may be a preferred model for studying the outcomes of myocardial infarction after coronary artery ligation, because a number of clinically relevant endpoints may be reproduced.

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