Chronic myocardial infarction in the mouse: cardiac structural and functional changes

Esther Lutgens, Mat J.A.P. Daemen, Ebo D. de Muinck, Jacques Debets, Peter Leenders, Jos F.M. Smits

Abstract

Objectives: We studied the effects of chronic left coronary artery ligation on cardiac structure and function in the mouse. Methods: Morphometric studies of the left ventricle were performed in coronary artery-ligated and sham-operated animals at one, two, three and five weeks after surgery. The fraction of DNA-synthesizing cells was determined as the fraction of cells incorporating 5'-bromo-2'-deoxyuridine, which was infused by osmotic minipumps one week before sacrifice. Collagen content of the septum was determined morphometrically. Left ventricular pressure and its derivatives were measured in separate groups of animals at one and three weeks after surgery. Results: Ligation of the main left coronary artery resulted in antero-apical infarction of the left ventricular wall, involving ~40% of left ventricular circumference. Infarction resulted in thinning of the infarcted area and left ventricular dilatation. DNA synthesis increased, peaking between one and two weeks in the border-zone of the infarct (22-fold), septum (ten-fold) and right ventricle (five-fold). At five weeks, DNA synthesis was still increased in the border zone of the infarct. Septal collagen content increased ~eight-fold in infarcted mice at two weeks, and decreased thereafter; it was still significantly elevated at five weeks. Left ventricular systolic pressure, and maximal positive and negative dP/dt decreased following infarction; left ventricular end-diastolic pressure was elevated at three weeks, but this effect was not statistically significant. Conclusion: These data provide basic information on changes in cardiac structure and function in mice following chronic coronary artery ligation. They indicate the feasibility of induction of chronic myocardial infarction in this species. Furthermore, they show the similarity of cardiac structural and functional consequences of chronic myocardial infarction in mice to those previously described in rats. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Myocardial infarction; Ventricular remodeling; Cardiac function

1. Introduction

The classical animal species for the study of cardiac remodeling and heart failure following myocardial infarction (MI) is the coronary artery-ligated rat. The rat MI model has provided a wealth of information on molecular, cellular, physiological and pharmacological data with respect to heart failure following MI [1].

Recent developments in gene-targeting and transgenic technologies make it possible to further analyze the contributions of endogenous systems to heart failure. However, development of transgenic rat models is technically very demanding and construction of null-mutants (knockouts) in rats has not been achieved at present. In contrast, developments in the mouse as a species for this type of technology are moving very rapidly. This has resulted in a growing interest in the mouse as a popular animal species for cardiovascular research, including heart failure.

* Corresponding author. Tel.: +31-43-388-1421; Fax: +31-43-367-0940; E-mail: j.smits@farmaco.unimaas.nl

See pages 506–508.

PII: S0008-6363(98)00216-8

0008-6363/99 – see front matter © 1999 Elsevier Science B.V. All rights reserved.

Time for primary review 32 days.
Some short-term studies on ischemia and reperfusion of the mouse heart have been reported [2,3]. Two studies, which focused on apoptotic events following ischemia [3] and the protective role of IGF-1 in myocardial ischemia [4], respectively, included mice at seven days following induction of ischemia. So far, however, basic information on the structural and functional adaptation of the murine heart to chronic myocardial infarction is lacking.

In the present study, we applied technologies that were originally developed in rats [5,6] to explore the feasibility of a chronic model for MI in mice, and to characterize both structural and functional aspects of cardiac adaptation during the first five weeks following infarction.

2. Methods

Male mice (Swiss, Iffa Credo), weighing 30–45 g (10–12 weeks old) at the time of surgery, were used. Animals had free access to standard lab-food and tap water. All experiments were performed according to institutional guidelines.

2.1. Surgical methodologies

2.1.1. Coronary artery ligation

Myocardial infarction (MI) was induced by coronary artery ligation. To this end, animals were anesthetized with pentobarbital (100 mg/kg i.p.), fixed in the supine position and the trachea was intubated with a 1.1-mm steel tube. Positive pressure respiration (1.5–2 ml, 70 strokes/min) was started and the left thorax was opened in the fourth intercostal space. All muscles overlying the intercostal space were dissected free and retracted with 5-0 silk threads; only the intercostal muscles were transected. After opening the pericardium, the main left coronary artery, which was clearly visible, was ligated just proximal to its main bifurcation [2], using 6-0 silk and an atraumatic and blocking of endogenous peroxidase, they were washed three times with 0.1 M PBS, were incubated with ABC (Brunschwig, Detroit, USA) 1:1250, v/v) for 30 min at room temperature. The sections were counterstained with hematoxylin.

2.1.2. Infusion of 5′-bromo-2′-deoxyuridine

Under ether anesthesia, all animals received 5′-bromo-2′-deoxyuridine (BrdU, Serva, Heidelberg, Germany; infusion rate 13 mg/kg/day) from an osmotic minipump (Alzet 2001, Alza Corporation, Palo Alto, CA, USA) seven days before sacrifice, to label DNA-synthesizing cells.

2.2. Structural measurements

2.2.1. Tissue processing

Under ether anesthesia, the animals were sacrificed at one, two, three or five weeks after surgery. Following cardiac arrest by injection of 1 ml of cadmium chloride (0.1 M) into the inferior vena cava, animals were retrogradely perfused through the aorta with phosphate buffered saline (PBS; pH 7.4) containing 1 mg/ml sodium nitroprusside over 3 min at a pressure of 100 mmHg, followed by 3 min of perfusion with a 5% solution of formalin in PBS at the same pressure. The heart was dissected, and the ventricles were weighed, immersion-fixed in 10% formalin in PBS over 24 h and subsequently cut transversely into two slices through the center of the infarct. The slices were embedded in paraffin according to routine histological procedures, and 4 μm sections were prepared.

2.2.2. Morphometry

All morphometric parameters were measured on two sections.

Infarct size was measured using a computerized morphometry system (Quantimet 570, Leica, The Netherlands) on AZAN-stained sections (cf. Fig. 1A–B) that were taken in the center of the infarct. The infarct size was measured as a percentage of the circumference of the left ventricle.

Left ventricular diameter was estimated from the morphometrically measured circumference of the left ventricle, assuming a perfectly circular shape (inner circumference/π). Average infarct thickness was calculated from the ratio of the morphometrically measured area of infarction and the average length of the infarcted area; average septal thickness was similarly calculated from the ratio of the area of septum and the average length of the septum [7].

2.2.3. Measurement of DNA synthesis

De-paraffinized sections were processed for anti-BrdU staining using a modification of an earlier published protocol [8,9]. Briefly, after dehydration of the sections and blocking of endogenous peroxidase, they were washed in tap water and digested in 0.02 mg/ml pepsin (Boehringer, Mannheim, Germany)/0.1 N HCl (30 min, 37°C). Sections were then incubated in 1.5 N HCl (15 min, 37°C) and washed in 0.1 M sodium tetraborate (pH 8.5). Incubation with a rat monoclonal anti-BrdU antibody, (1:20,000 1 h, 37°C) was followed by incubation with a biotin-labeled sheep anti-mouse IgG (Amersham Life Sciences, UK; 1:1250, v/v) for 30 min at room temperature. The sections were incubated with ABCPO (Brunschwig, Detroit, USA) for 30 min. As a chromogen, we used 3,3′-diaminobenzidine (DAB). Sections were counterstained with hematoxylin and mounted with coverslips (Fig. 1C–D).

The total labeling fraction (LF=number of BrdU-positive/total number of counted nuclei×100%) was calculated in the border regions of the infarcted left ventricle, the center of the non-infarcted right ventricle and in center of the non-infarcted septum. Cell numbers were determined microscopically with an eyepiece grid (400×...
magnification) as described previously [8]. A total of 4000 nuclei per heart were counted in two tissue sections (2000 at the borders of the infarcted left ventricle, 1000 in the center of the non-infarcted right ventricular wall and 1000 in the center of the non-infarcted septum, all in the same section). All measurements were performed by one investigator (EL). Intraobserver variation was less than 10%. The investigator was blinded for the experimental group.

2.2.4. Measurement of collagen amount
Deparaffinized sections were incubated for 5 min with 0.2% aqueous phosphomolybdic acid [10] and then incubated for 90 min with 0.1% Sirius red F3BA (C.I 35780, Polysciences, Northampton, UK) in saturated aqueous picric acid, and washed for 2 min with 0.01 M HCl. They were then dehydrated and mounted with coverslips [11].

The relative collagen area of the non-infarcted septum, i.e. the percentage of total area that stained positive for Sirius red (Fig. 1E–F), was determined under a microscope coupled to a computerized morphometry system (Quantimet 570, Leica). Twenty-four fields (magnification, 400×) in two tissue sections were analyzed in the midwall

Fig. 1. Typical histological sections as obtained in the present study. (A) and (B) are AZAN-stained sections of an infarcted and a sham-operated animal, respectively; the infarcted area stains blue. (C) and (D) are BrdU-stained sections of an infarcted (C) and a sham-operated (D) heart; BrdU-positive nuclei stain brown. (E) and (F) are Picrosirius red-stained sections of an infarcted (E) and a sham-operated (F) heart; in these sections, collagen stains red.
area of the septum. Measurements were restricted to the interstitial collagen; perivascular and endocardial collagen were excluded from the measurements.

2.3. Functional measurements

2.3.1. Left ventricular pressure measurement

After one or three weeks following MI or sham surgery, animals were re-anesthetized with pentobarbital (110 mg/kg i.p.) and the trachea was intubated to facilitate spontaneous breathing. A rectal thermocouple was used to continuously monitor body temperature, which was maintained at 37°C using a heating-pad. The skin in the neck was opened and the right carotid artery was isolated. Two 6-0 silk ligatures were looped around it and a small incision was made, through which, a catheter tip pressure transducer (Mikro tip 1.4F; Millar Instruments, Houston, TX, USA) was inserted. The transducer was advanced into the left ventricle under guidance of the pressure signal and fixed, when in position. Care was taken to avoid any blood loss.

Left ventricular pressure was monitored for 10 min; data were sampled with a computer at a rate of 2 kHz, and stored for later analysis of left ventricular systolic pressure (LVSP), end-diastolic pressure (LVEDP) and maximal positive (+dP/dt) and negative (−dP/dt) rates of pressure development.

2.4. Statistics

Data are expressed as means±SEM. The effects of surgery and time were evaluated in a two-way analysis of variance. If this indicated a statistically significant effect, groups were compared by a Bonferroni test for multiple comparisons. The level of statistical significance was assumed to be at P<0.05.

3. Results

3.1. General

A total of 48 animals were subjected to sham surgery; 46 (96%) survived and were included in the experiments. Survival in the infarcted group was lower; a total of 91 labeling of cardiomyocytes (Table 1). In none of the sham-operated animals did we observe infarctions.

LV diameter (Fig. 2) in sham-operated animals was stable from one week (2.1±0.1 mm) to five weeks after surgery (1.7±0.2 mm). In infarcted mice, LV diameter was increased at one week (3.0±0.3 mm) and slight, but statistically insignificant further dilatation occurred over time, to 3.6±0.3 mm at five weeks (Fig. 2).

Sham-operated animals had an average LV wall thickness of 1.5 mm, which did not change over time (Fig. 2). MI resulted in thinning of the LV free wall, from 0.7±0.3 mm at one week, to 0.5±0.3 mm at five weeks. In contrast, the average septal thickness was similar in sham-operated and MI mice; only at three weeks after MI was there a slight, but significant, increase in the average septal thickness.

3.2. Structural measurements

3.2.1. Morphometry

Coronary artery ligation resulted in infarcts of 44.6±2.1% of the LV circumference. There were no differences between the groups sacrificed at different times (Table 1a). In none of the sham-operated animals did we observe infarctions.

LV diameter (Fig. 2) in sham-operated animals was stable from one week (2.1±0.1 mm) to five weeks after surgery (1.7±0.2 mm). In infarcted mice, LV diameter was increased at one week (3.0±0.3 mm) and slight, but statistically insignificant further dilatation occurred over time, to 3.6±0.3 mm at five weeks (Fig. 2).

Sham-operated animals had an average LV wall thickness of 1.5 mm, which did not change over time (Fig. 2). MI resulted in thinning of the LV free wall, from 0.7±0.3 mm at one week, to 0.5±0.3 mm at five weeks. In contrast, the average septal thickness was similar in sham-operated and MI mice; only at three weeks after MI was there a slight, but significant, increase in the average septal thickness.

3.2.2. DNA synthesis

Following MI, BrdU-incorporation increased in all parts of the ventricles. The majority of positive nuclei were located in interstitial cells, although there was also definite labeling of cardiomyocytes (<10% of positive nuclei).

MI caused a transient increase in BrdU-incorporation in the border zone of the infarct (Fig. 3). Peak levels were observed at one week after surgery (2.0±0.6% in sham-operated vs. 45.0±5.5% in MI animals). At five weeks after MI, DNA synthesis had returned to a lower level, but was still significantly elevated in MI animals (2.5±1.7% in sham-operated vs. 10.1±5.2% in MI animals).

Increased DNA synthesis was also observed in the non-infarcted septum and right ventricle (Fig. 3). Peak levels occurred at two weeks and were 14.8±3.3% in the right ventricle and 17.6±6.1% in the septum. At five
weeks after MI, DNA synthesis in the non-infarcted areas had returned to sham levels.

3.2.3. Collagen content

The Sirius Red positive area in the septum was increased after MI, throughout the five-week period (Fig. 4). Peak levels were observed at two weeks (0.8 ± 0.1% in sham-operated vs. 6.2 ± 0.9% in MI animals). Thereafter, the collagen content decreased, but remained significantly elevated (Fig. 4).

3.3. Hemodynamic measurements

Table 1b summarizes body weights and heart weights
was significantly reduced. Impairment of left ventricular contraction and relaxation was apparent from reductions of maximal positive and negative \( \frac{dP}{dt} \), respectively. At one week post-surgery, LVEDP was similar in sham and MI mice (sham, 5.3±0.8 and MI, 5.3±1.2 mmHg, respectively). At three weeks post-MI, LVEDP was increased in MI compared to sham-operated mice (sham, 3.5±0.5 and MI, 4.9±0.6 mmHg); the difference was, however, not significant \( (P=0.10) \).

4. Discussion

Genetic modification is frequently used to elucidate the function of endogenous substances. The standard animal species in which such genotypical modifications are being performed is the mouse. However, phenotypical characterization is lagging behind the abilities to manipulate the genotype. In the present study, we quantified structural and functional consequences of chronic ligation of the left main coronary artery in Swiss mice. We obtained evidence for LV remodeling, including LV dilatation and hypertrophy. Interstitial DNA synthesis and collagen deposition increased throughout the heart. These structural changes were associated with decreased \( \frac{dP}{dt} \), although LVEDP did not significantly increase. Our results indicate the feasibility of coronary artery ligation for inducing chronic myocardial infarction in mice, and provide basic data on the ensuing cardiac structural and functional adaptations in this species.
Coronary artery ligation as a method to induce myocardial infarction has been extensively documented in rats [1,5,6,12]. In rats, the procedure results in gross transmural infarctions of the left ventricular free wall, which may encompass up to 60% of the total left ventricular circumference. Left main coronary artery ligation in mice similarly results in large infarcts that do, however, differ anatomically from those in rats. As in rats, infarcts are, in general, transmural and do not involve the interventricular septum and right ventricle. Typically, however, infarcts from this procedure in mice are located antero-apically, as opposed to LV free wall infarcts in rats. This probably relates to differences in coronary anatomy between mice and rats [2].

Infarcted mice displayed marked LV wall thinning. At one week post-MI, the LV wall thickness was reduced to approximately 50%, compared to that in sham-operated animals; at five weeks post-MI, we measured a wall thickness of only 0.5 mm, compared to 1.5 mm in sham-operated mice. As opposed to what we normally observe in rats, mice invariably exhibited aneurysm of the infarct, which is probably related to the extreme thinning, at comparable operating blood pressures in the two species. Rupture of the aneurysm was never observed; in animals that died after the initial peri-surgical drop-out (<5%), we performed an autopsy, whenever possible, and never observed a hemorhorax.

The loss of viable left ventricular myocardium induces dilatation of the left ventricular cavity, which results from side-to-side slippage of the cardiomyocytes in the surviving myocardium [13]. In our mice, we observed an approximate doubling of LV diameter, which is consistent with such dilatation. LV dilatation has been suggested to be one of the triggers for LV hypertrophy; thickening of the LV wall may reduce the increased wall stress that results from dilatation. Although LV hypertrophy in mice following MI was evident from maintenance of cardiac weight, in spite of excessive thinning of the large infarcted area, we did not consistently observe thickening of the interventricular septum, suggesting eccentric hypertrophy. This implies that thickening of the LV wall does not contribute to the reduction of increased wall stress in mice following MI. Increased wall stress as such has been proposed to be the trigger for cardiomyocyte hypertrophy and the consequent increase in wall thickness. The reasons for this lack of increase in wall thickness remain to be established.

In rats, we have previously observed increased interstitial DNA synthesis in the surviving myocardium following MI [8]. Overall, the kinetics and magnitude of the response in the rats and mice were comparable, i.e. a five–ten-fold increase in DNA synthesis around one week and a return to baseline levels at five weeks in the non-infarcted area [8]. In rats, increased DNA synthesis was exhibited mainly by fibroblasts, whereas endothelial cell DNA synthesis contributed approximately 30% to the overall increase [14]; less than 1% of BrdU-positive cells were cardiomyocytes [8]. Although we did not attempt characterization of the BrdU-positive cells in mice, clearly, in that species, more cardiomyocytes were involved, which may constitute a difference between the species.

Also, with respect to interstitial fibrosis, the rat and mouse heart respond in a qualitatively similar manner. Both species exhibit a strong increase in collagen content, which is maximal at one–two weeks after MI [15]. In rats, increased collagen is maintained at a constant elevated level for at least 90 days after MI, which is compatible with the long-lasting increase that we have observed in human hearts following MI [16]. In our mice, we observed a rapid decline after the initial peak, although collagen was still significantly elevated at five weeks after MI. This suggests a relatively rapid turnover of collagen in the mouse, which may depend on a transient increase in synthesis or rapid degradation.

The relative loss of collagen might, hypothetically, contribute to ventricular dilatation. However, the relatively small change in left ventricular diameter between two and five weeks does not substantiate that hypothesis in the light of the large decrease in collagen content over that period, although, obviously, only additional qualitative assessment of the collagen network can rule it out.

In the present study, we observed decreases in left ventricular systolic pressure and maximal positive and negative rates of pressure development. These observations are consistent with diminished contractile function. In contrast, LVEDP did not change significantly, which suggests a lack of circulatory congestion. Again, similar observations have been made in rats; LVEDP was found to be increased only in rats with very large infarcts [5].

The values for the rate of pressure development in the mouse heart in the present study are compatible with those in previous studies [17–19], but lower than those in recent studies in closed chest mice [4,20]. Similarly, our values for LVEDP in sham-operated mice were lower than those in the latter studies [4,20]. This may relate to the fact that we used a smaller pressure transducer (1.4F versus 2F [20] and 1.8F [4]), thereby reducing the risk of aortic regurgitation. Alternatively, differences may depend upon the anaesthetics used, or strain differences with respect to hemodynamics [21].

In conclusion, our studies provide background data with respect to the nature and kinetics of the cardiac structural and functional response to chronic myocardial infarction. The data indicate a high degree of similarity, suggesting that the mouse is equally acceptable as the rat as a model for chronic myocardial infarction. Studies with genetically modified mice are now underway.

References


