Original Research

Vascular rhexis: loss of integrity of coronary vasculature in mice subjected to myocardial infarction

Christopher J French, A K M T Zaman, Robert J Kelm Jr, Jeffrey L Spees and Burton E Sobel

Department of Medicine, Cardiovascular Research Institute, University of Vermont, 208 South Park Drive, Colchester, VT 05446, USA
Corresponding author: Dr Burton E Sobel. Email: Burton.Sobel@uvm.edu

Abstract

We previously observed gross hemorrhage in plasminogen activator inhibitor type-1 (PAI-1) knockout (PKO) mice with induced myocardial infarction (MI). We hypothesized that it reflected degradation of vessels – a phenomenon we termed vascular rhexis. Accordingly, in the present study we characterized vascular rhexis in C57BL6 mice. MI was induced in 10- to 12-week-old mice by coronary artery ligation for 24, 48, 72 or 96 h. Hemorrhage was quantified by non-cross-reacting enzyme-linked immunosorbent assay of left ventricular (LV) hemoglobin corrected for myoglobin. Degradation of vasculature was quantified by the appearance of alpha smooth muscle actin (αSMA) in low salt soluble fractions of LV homogenates (Western blotting) and by immunohistochemistry (residual αSMA). Co-staining for CD31 (endothelial cells) and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) (a marker of cell death) was used to identify capillary rhexis. PKO mice ($n = 9$) had marked hemorrhage in infarct zones (432 ± 27 standard error of mean μL blood/g). Hemorrhage was evident in C57BL6 mice as well ($n = 6$): 51 ± 8 μL/g LV 96 h after coronary occlusion compared with 10 ± 5 μL /g, $n = 13$ in normal LVs. Residual intact vasculature was reduced 48 h after infarction. Thus, an average of 16 ± 1.6 small- and medium-sized vessels ($n = 5$ hearts) were seen compared with 84 ± 4.8 in normal LVs ($n = 3$, $P \leq 0.05$). An approximately three-fold increase in soluble αSMA 48 h after MI (2.68 ± 0.28, $n = 6$) was seen relative to that in normal LVs defined as 1.0 ± 0.04, $n = 10$, $P \leq 0.05$. Capillary degradation was evident as well, as judged from CD31 and TUNEL co-localization. Vascular rhexis occurs within 48 h after the onset of MI. It may contribute to the early no-reflow phenomenon and to late negative LV remodeling.

Keywords: myocardial infarction, vascular rhexis, PAI-1, remodeling, ischemia, heart failure, microcirculation, necrosis

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Introduction

Coronary artery disease precipitating acute coronary syndromes (ACS) is a major cause of long-term morbidity and mortality.[1] The current paradigm for treatment of acute myocardial infarction (MI) is rapid restoration of coronary artery patency and reduction of myocardial oxygen requirements. However, 30-day mortality remains as high as 10%. In addition, late mortality associated with heart failure secondary to left ventricular (LV) negative remodeling is common.[2–6]

We previously noticed that PAI-1 knockout (PKO) mice congenic on a C57BL6 background frequently exhibited grossly hemorrhagic-induced infarctions.[7] This led us to believe that intensified fibrinolysis secondary to the absence of plasminogen activator inhibitor type-1 (PAI-1) was unmasking a loss of integrity of coronary arterioles – a phenomenon that we termed vascular rhexis.[8]

A phenomenon called no-reflow associated with MI is well recognized.[9–11] Myocardial reperfusion can be markedly reduced despite prompt re-canalization of macroscopic coronary arteries by primary percutaneous coronary intervention.[9–12] Such 'no-reflow' is thought to be caused by a combination of distal embolization, platelet aggregation, platelet leukocyte plugs, endothelial cell swelling and susceptibility of the coronary microcirculation to injury mediated by cytokines and other moieties.[9,13,14] To the best of our knowledge, however, degradation of the coronary vasculature *per se* as a cause of no-reflow or negative remodeling has not been described previously. The objective of the present study was to determine whether vascular rhexis occurs in normal mice and, if so, to characterize the time course of its evolution after induction of ischemia.

Methods

Experimental animals

Genetically modified mice rendered PAI-1 deficient and congenic on a C57BL6 background and normal C57BL6 controls (from Jackson Laboratory, Bar Harbor, ME, USA) were used in conformity with a protocol approved by the University of Vermont Institution Animal Care and Use Committee and with adherence to the NIH Principles of Animal Care. The mice were fed normal standard laboratory rodent chow and given water *ad libitum*. At 10 weeks of age, PKO and C57BL6 mice were subjected to either sham operation (controls) or coronary occlusion. Hearts from PKO mice were harvested 48 h after coronary ligation, and those from C57BL6 mice were harvested at 24, 48, 72 and 96 h after ligation.

Surgical procedures

Acute MI was induced in 10-week-old mice as described previously.[7] Through an 8 mm skin incision from the left sternal border at the fourth intercostal space, the pericardium was removed and the left anterior descending coronary artery (technically the middle left in mice) was identified after retraction of the left atrium. The artery was ligated with the use of 8-0 suture on a tapered needle 2 mm below the tip of the left atrial appendage. Occlusion was confirmed by blanching of LV myocardium below the suture. For animals undergoing a sham operation, a ligature was placed in a corresponding location but not tied. The chest cavity was then closed in layers, and the animals were allowed to recover.

Drug treatment of PKO mice

PKO mice were treated with aprotinin to inhibit plasmin and reverse the functional consequences of the fibrinolytic system deficiency and with cyclophosphamide to attenuate inflammation. We previously observed a large inflammatory LV response to MI in PKO mice, presumably reflecting enhanced cellular mobility mediated by unopposed cell surface expression of plasminogen activators. Nine PKO mice were subjected to MI, but not given either agent. Twelve PKO mice were given cyclophosphamide (150 mg/kg/dose intraperitoneally, with the first dose given 72 h before coronary occlusion and the second given immediately before coronary occlusion to constrain inflammation). Aprotinin (5000 KIU/dose, $n = 4$) was given to PKO mice 12 and 6 h before harvest of tissue. Four PKO mice were given both agents.

Harvesting of tissue

Mice in each group were killed humanely at selected intervals as described previously. Blood samples were drawn from the right ventricle of PKO mice for determination of the white blood cell count in whole blood with the use of a hemocytometer (Hausser Scienitifc, Horsham, PA, USA). After perfusion with phosphate-buffered saline (PBS) *in situ*, the hearts were excised. The LVs were separated from the right ventricles and atria and weighed. In some animals the infarct zone was separated from the rest of the left ventricle.

Assessment of the extent of infarction by assay of residual LV creatine kinase

LV myocardial creatine kinase (CK) content was determined as follows: the mice were killed humanely under 4% isofluorane anesthesia, the hearts excised and stored at -80°C. Homogenization of the LVs was performed with homogenization buffer containing 50 mmol/L Tris, 5 mmol/L dithiothreitol, at 4°C. The pH was adjusted to 7.6 with HCl with a ratio of homogenization buffer to tissue weight of 2.0 mL/100 mg. CK and protein assays were performed as described previously.[15] The extent of infarction (percent) was calculated from the following equation: $([8.1 - \text{CK(IU/mg protein)}/6.7) \times 100$, in which CK is the measured total LV CK/mg protein, 8.1 ± 0.6 IU/mg is the amount in normal LV and 1.4 ± 0.1 IU mg is the amount in homogeneous zones of infarction.

Quantification of LV hemorrhage

LV hemorrhage was quantified based on assays of LV extracts by enzyme-linked immunosorbent assay (ELISA) for hemoglobin corrected for myoglobin measured with a non-cross-reacting ELISA. These assays were performed with the use of the QuantiChrom Hemoglobin Assay Kit (DIHB-250) from BioAssay Systems (Hayward, CA, USA) and a Myoglobin (Mouse) ELISA kit, Catalog Number 41-MYOMS-E01 from ALPCO (Salem, NH, USA).

Histology and immunohistochemistry

Five-μm-thick LV sections from hearts of PKO mice subjected to infarction with or without administration of cyclophosphamide were stained with hematoxylin and eosin for characterization of inflammatory cell infiltrates in myocardium. Immunohistochemical staining for alpha smooth muscle actin (αSMA) was performed with 5 μm paraffin sections obtained from the LVs following their excision and immersion into 3% formaldehyde (prepared freshly from paraformaldehyde) in PBS overnight at 4°C. The sections were de-paraffinized in xylene and re-hydrated through an ethanol series. Antigen retrieval was performed with DAKO Target Retrieval Solution (#S1699, Dako, Carpenteria, CA, USA) diluted 1:10 with distilled water for 45 min at a temperature of 95–99°C. After being washed with PBS/1% bovine serum albumin (BSA) slides were incubated overnight at 4°C with monoclonal anti-αSMA Cy3 conjugate (1:300 dilution with PBS/1% BSA, Sigma, Saint Louis, MO, USA). They were then rinsed with two changes of PBS/1% BSA, 5 min each, in the dark and counterstained and mounted with Vectashield with 4′,6-diamidino-2-phenylindole (H-1200, DAPI, Vector Laboratories Inc, Burlingame, CA, USA) for preservation of fluorescence and visualization of cell nuclei.

For terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) and immunostaining of CD31, 10 μm cryostat sections were

obtained from the LVs after fixation with 3% formaldehyde in PBS overnight at 4°C. Slides were rinsed twice with PBS/1% BSA and were blocked with 10% normal goat serum (#191356, MP Biomedicals LLC, Solon, OH, USA) diluted in PBS/1% BSA for 30 min. They were then incubated with purified rat anti-mouse CD31 primary antibody (1:40 dilution with PBS/1% BSA, #550274, BD Bioscience, San Jose, CA, USA) overnight at 4°C. After being washed with PBS/1% BSA, they were treated with goat anti-rat ALEXA-594 (Invitrogen), diluted 1:600 in PBS/1% BSA followed by rinsing in two changes of PBS/1% BSA, 5 min each in the dark. Sections were then stained for TUNEL with the use of Apoptag Plus S7111 fluorescence *in situ* detection kits (Chemicon International, Temecula, CA, USA) to detect necrotic cells.[16] Slides were counterstained and mounted with Vectashield with DAPI. Photographs were taken with the use of an epifluorescence microscope equipped with an automated x, y and z stage for de-convolution (Leica DM6000B microscope, Leica CCD camera and Leica Deblur de-convolution software, Leica Microsystems, Bannockburn, IL, USA). For quantification of αSMA in the infarct zone, the total number of small- to medium-sized vessels of less than 100 μm in diameter from three separate sections were counted and averaged. In a corresponding region of equal size, three sections were used for quantification of the number of vessels in normal LVs. The number of vessels was counted independently by two different observers blinded to each other's results.

Assay of soluble αSMA released from the infarct zone detected by Western blotting of low salt soluble fractions of LV homogenates

To characterize the evolution of the phenomenon of vascular rhexis, the presence and amounts of αSMA in the soluble fraction of LV extracts that we hypothesized would reflect release of αSMA from vascular smooth muscle cells (VSMCs) undergoing degradation was determined by Western blotting. Our approach is predicated on the concept that as VSMCs undergo cell death, αSMA is liberated and rendered soluble and consequently appears in low salt soluble fractions of LV extracts. Protein concentrations were determined with the use of the Bradford assay[17] and 5 μg of protein was loaded into each well. Coomassie staining was used to confirm protein loading. Samples were incubated with the primary antibody, monoclonal anti-αSMA clone 1A4 (#A2547, Sigma) for two hours at room temperature on a rocking platform followed by treatment with the secondary antibody, goat anti-mouse immunoglobulin G-horse radish peroxidase (sc-2005, Santa Cruz Biotechnology) for one hour in the dark on a rocking platform. Detection was performed with the use of Pierce ECL Western Blotting Substrate (#32209, Thermo Scientific, Rockford, IL, USA) and quantified by chemiluminescence. Results were expressed as values relative to those seen in LVs from normal mice. In each case, normal tissue and tissue from hearts undergoing infarction were compared on the same gel.

Statistical analysis

Results were expressed as means ± standard error of mean (SEM). Data were analyzed by analysis of variance or Student's t-test. Variables that were not normally distributed were compared with the use of Kruskal–Wallis tests. Significance was assumed to be present when $P \leq 0.05$.

Results

Myocardial hemorrhage in PKO mice

In the absence of hemorrhage, the LV wall contains blood essentially only in the vasculature (approximately 10–40 μL/g LV). As seen in Figure 1, PKO mice ($n = 9$) had

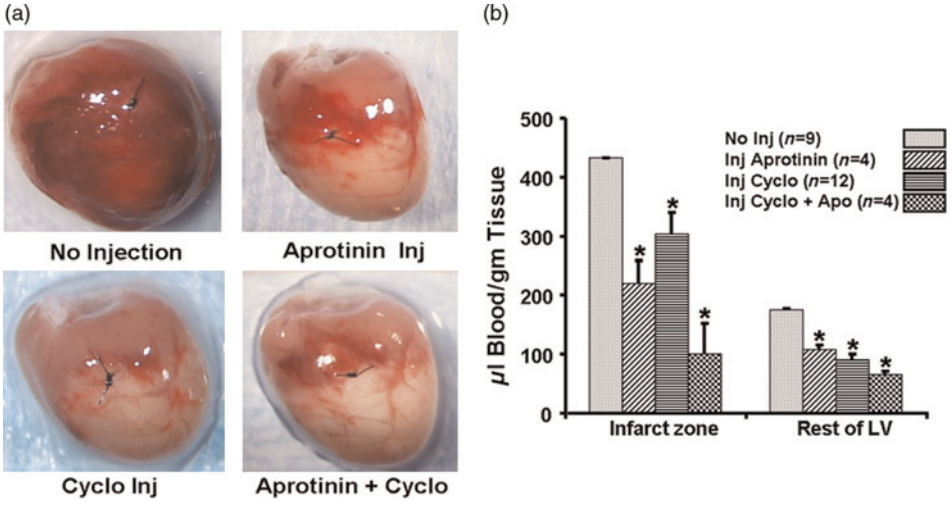

Figure 1 Hemorrhage in hearts of plasminogen activator inhibitor type-1 knockout (PKO) mice. (a) Representative photographs of hearts from PKO mice subjected to infarction. PKO mice exhibit grossly hemorrhagic infarcts 48 h after coronary occlusion as shown in the top left panel. Treatment with aprotinin or cyclophosphamide either alone or in combination markedly reduced hemorrhage in the infarct zone 48 h after coronary occlusion. (b) Quantification of the amount of hemorrhage in the LVs of PKO mice subjected to selected intervals of persistent ischemia. Values are expressed as means ± standard error of mean; *significantly different from results in LVs from normal mice ($P \leq 0.05$)

marked intramyocardial hemorrhage in infarct zones (432 ± 27 μL/g). The hemorrhagic diathesis was evident as early as 48 h after infarction. Hemorrhage was significantly reduced (302 ± 37 μL/g, $P < 0.05$) by treatment with cyclophosphamide. Cyclophosphamide induced profound leukopenia (Figure 2) and obviated the appearance of inflammatory cells in the heart seen otherwise (Figure 3). Aprotinin (5000 KIU/dose, $n = 4$) reduced hemorrhage significantly as well (220 ± 40 μL/g, $P < 0.05$). The combination of both ($n = 4$) virtually eliminated hemorrhage (101 ± 52 μL/g, $P < 0.05$), although infarct size was not affected (PKO: $46 \pm 2\%$ of LV mass; PKO with cyclophosphamide: $45 \pm 1\%$; PKO with aprotinin: $43 \pm 2\%$) (Figure 1).

Hemorrhage in mice without PAI-1 deficiency

We sought to determine whether the loss of vascular integrity occurred in mice with normal fibrinolytic systems even though we anticipated that the hemorrhagic diathesis would be much less intense than it was in PKO mice. A modest amount of hemorrhage (51 ± 8 μL/g LV, $n = 6$) was seen in C57BL6 mice in the whole LV that became evident 96 h after coronary occlusion compared with 10 ± 5 μL/g ($n = 13$) that was present 30 min to four hours after coronary occlusion (Figure 4).

Vascular rhexis in C57Bl6 mice

Vascular rhexis was evident in C57BL6 mice subjected to MI as judged from the increase in soluble αSMA (Figure 5). The amount of αSMA in homogenates of LV was similar 24 h after MI (1.07 ± 0.04, mean $\pm$ SEM, $n = 3$) relative to that seen in normal hearts before MI (1.0 ± 0.05, $n = 10$). Peak αSMA was seen 48 h after MI (2.68 ± 0.28, $n = 6$) and was significantly greater ($P \leq 0.05$) than that seen before MI or 24 h after MI. The αSMA content in low salt soluble fractions declined at 72 h after MI, but was still significantly greater than that seen before MI or 24 h after MI (2.32 ± 0.45, $P \leq 0.05$). To further confirm that αSMA was being liberated from the infarct zone and not from the rest of the LV, αSMA was quantified in both regions in a set of animals that were subjected to infarction. Forty-eight hours after the induction of MI, there was significantly more αSMA in the infarct zone relative to that seen in the rest of the LV from the same heart (3.68 ± 1.63 in the infarct zone compared with 1.0 ± 0.29 in the rest of the LV, $n = 5$, $P \leq 0.05$; Figure 6). This dichotomy was seen also 72 h after the induction of MI (2.19 ± 0.53 compared with 1.0 ± 0.14, $n = 8$, $P \leq 0.05$).

To further confirm the existence of vascular rhexis, immunohistochemical assays of LV αSMA were performed. There was a marked reduction in the number of small arteries and arterioles in the infarct zone 48 h after the onset of coronary occlusion (Figure 7). There were 16 ± 1.6 small- and medium-sized vessels in the infarct zone compared with 84 ± 4.8 small- and medium-sized vessels in an equivalent area in normal hearts (Table 1). Further degradation of vessels was seen at 72 h (8.1 ± 2.3 small- and medium-sized vessels).

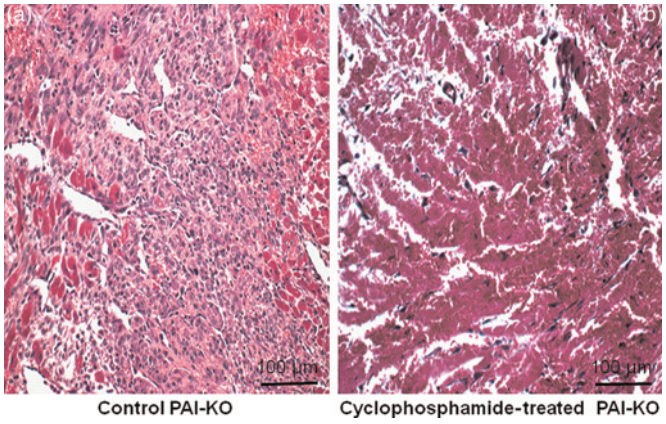

Figure 3 Effect of cyclophosphamide on the inflammatory response to myocardial infarction in hearts of plasminogen activator inhibitor type-1 knockout (PAI-KO) mice. Representative hematoxylin and eosin sections of LVs from PKO mice 48 h after the onset of ischemia. (a) A marked inflammatory response in the infarct zone. (b) A significant reduction in the number of inflammatory cells in the infarct zone from a mouse given cyclophosphamide

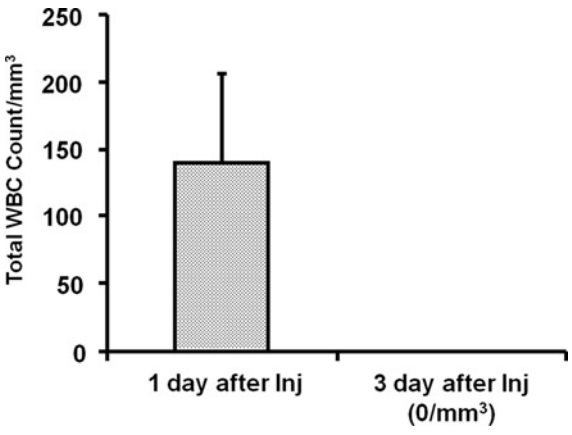

Figure 2 Effect of cyclophosphamide on white blood cell (WBC) counts in plasminogen activator inhibitor type-1 knockout (PKO) mice. Total WBC count in whole blood from PKO mice after injection of cyclophosphamide. Cyclophosphamide induced profound leukopenia

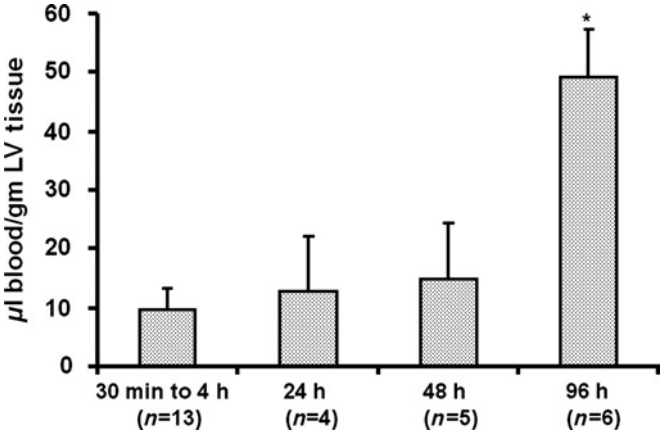

Figure 4 Hemorrhage in LVs of C57BL6 mice subjected to infarction. Results are means $\pm$ standard error of mean; *significantly different from results in the group with 30 min to four hours of persistent ischemia ($P \leq 0.05$)

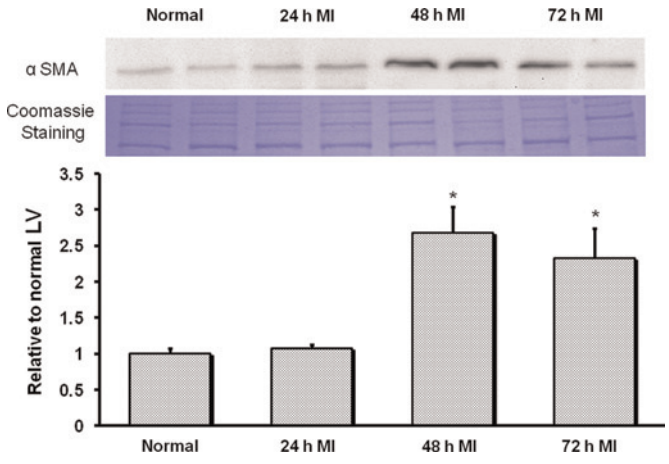

Figure 5 Low salt soluble alpha smooth muscle actin in homogenates from whole LVs. Amounts of low salt soluble alpha smooth muscle actin (αSMA) in LV homogenates from C57BL6 mice subjected to infarction (above). Quantification of αSMA 24, 48 and 72 h after coronary occlusion (below). Results are means $\pm$ standard error of mean; *significantly different from results in normal C57BL6 mice with no ischemia or with ischemia of only 24 h duration ($P \leq 0.05$). Coomassie staining demonstrates comparable protein loading

Analysis of loss of capillaries was performed with double staining for CD31, a marker of endothelial cells, and TUNEL, a marker of necrosis. A large percentage of cells in the infarct zone stained positively for both CD31 and TUNEL 48 h after MI (Figure 8). Although there were many cells that stained positively for TUNEL 24 h after MI, there was no co-localization of CD31 and TUNEL. The cells that stained positive for TUNEL were mostly cardiomyocytes. By 72 h after the onset of ischemia, normal capillary architecture was no longer present in the infarct zone.

Time course of cardiomyocyte cell death compared with that of vascular rhexis

Cardiomyocyte death reflected by loss of LV CK is evident four hours after coronary occlusion, and peak loss occurs within 72 h of the onset of ischemia (Figure 9). Vascular rhexis is evident after 48 h of persistent ischemia, and the extent of VSMC necrosis decreases after this time as shown in Figures 4–7.

Discussion

Our results demonstrate that a loss of integrity of coronary vasculature occurs beginning within 48 h after coronary occlusion in C57BL6 mice. The disintegration of coronary vasculature as a result of ischemia results in the progressive loss of integrity of arterioles and capillaries in the infarct zone. This is manifested by an increase in soluble αSMA in low salt homogenates of LVs and as a reduction in immunohistochemical staining of αSMA-positive vessels in the infarct zone. In addition, a loss of capillaries as a result of prolonged ischemia was demonstrated by co-localization of CD31, a marker of endothelial cells, and TUNEL, a marker of necrosis, by immunohistochemistry 48 h after coronary occlusion. In aggregate our data demonstrate that 48 h after MI, there is a loss of integrity of arterioles and capillaries in the infarct zone, i.e. vascular rhexis.

We considered the possibility that the appearance of αSMA in the low salt soluble fractions of LV homogenates may have reflected liberation from myofibroblasts rather than exclusively from vessels undergoing rhexis. However, this seems unlikely for the following reasons: (1) the presence of such cells in infarct zones is not evident until four

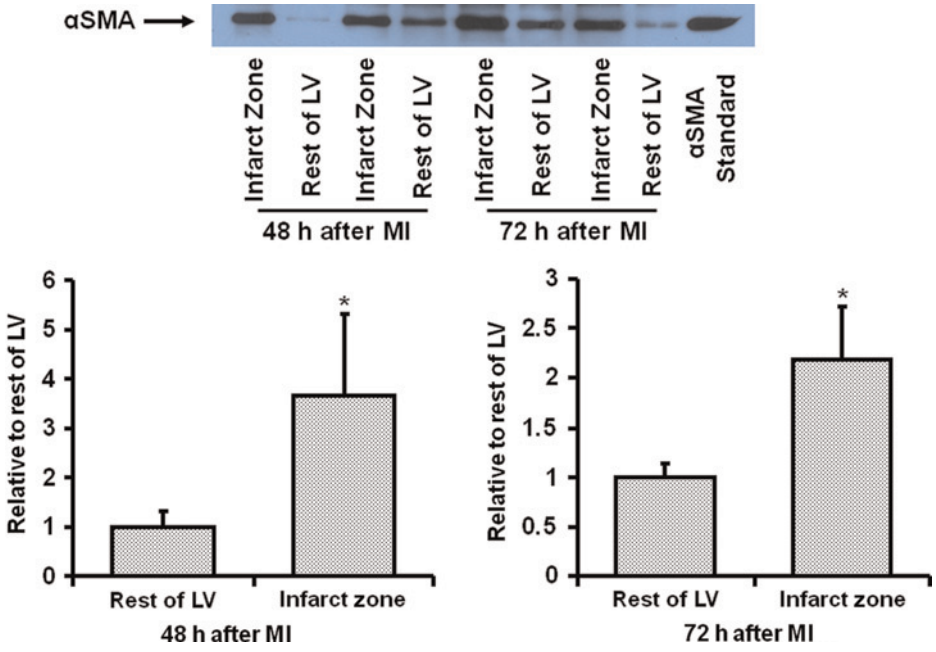

Figure 6 Low salt soluble alpha smooth muscle actin (αSMA) in homogenates from infarct zones and the rest of the LVs. Amounts of low salt soluble αSMA in homogenates from infarct zones and from the rest of the LVs from C57BL6 mice subjected to infarction (above) and quantification of αSMA 48 and 72 h after coronary occlusion are shown. Results are means $\pm$ standard error of mean; *significantly different from results in the rest of the LVs ($P \leq 0.05$)

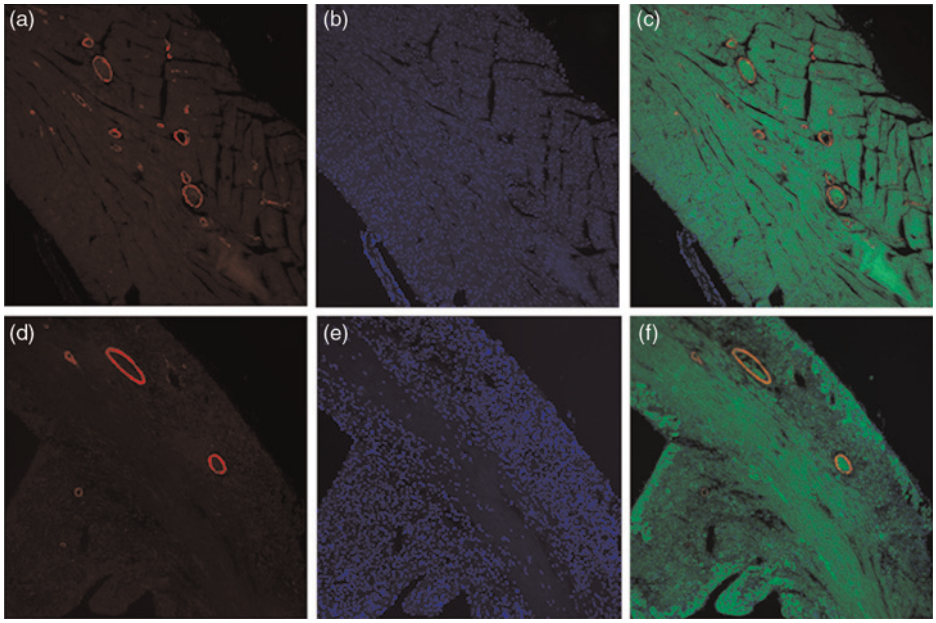

Figure 7 Immunohistochemical staining of alpha smooth muscle actin (αSMA). Immunohistochemical staining of αSMA in a normal heart (a–c) and a heart subjected to 48 h of ischemia (d–f). Staining for αSMA is shown in (a) and (d). The merged images are shown in (c) and (f). Staining for DAPI is shown in (b) and (e). The green cast in (c) and (f) is attributable to autofluorescence

Table 1 αSMA in LVs assayed immunohistochemically

	Small and medium vessels (<100 μm)
No MI	84.3 ± 4.75 ($n = 3$)
48 h MI	16.0 ± 1.6 ($n = 5$)*
72 h MI	8.1 ± 2.3 ($n = 5$)*

αSMA, alpha smooth muscle actin; LV, left ventricular; MI, myocardial infarction; SEM, standard error of mean
Number of small- and medium-sized vessels in the infarct zones in the numbers of mice indicated and in an equivalent area of LV from a mouse with no MI. Values are means ± standard error of mean
*Significantly different from results in hearts with no MI

days or more after the onset of an ischemic insult;[18] (2) the immunochemistry we performed did not demonstrate αSMA in loci other than vessels; (3) it is well known that elaboration of fibrous tissue in zones of infarction occurs relatively late. This accounts in part for the maximal frequency of ventricular rupture in patients being within 10 days after the onset of infarction in the absence of fibrinolysis; and (4) our studies of the time course of occurrence of hemorrhage indicate that vascular integrity is compromised in the same early time frame as that in which we observed augmentation of concentrations of αSMA in low salt soluble fractions of LV homogenates.

Deleterious effects have been attributed to hemorrhage after coronary re-canalization.[19] However, the extent and time course of vascular degradation underlying hemorrhage have not been established. There is a paucity of data relevant to the effects of ischemia and or reperfusion on the survival of vessels in the infarct zone.[20] However, extensive indirect evidence suggests that the degradation of vessels augments cardiomyocyte cell death and negative LV remodeling after MI followed by reperfusion.[9–12]

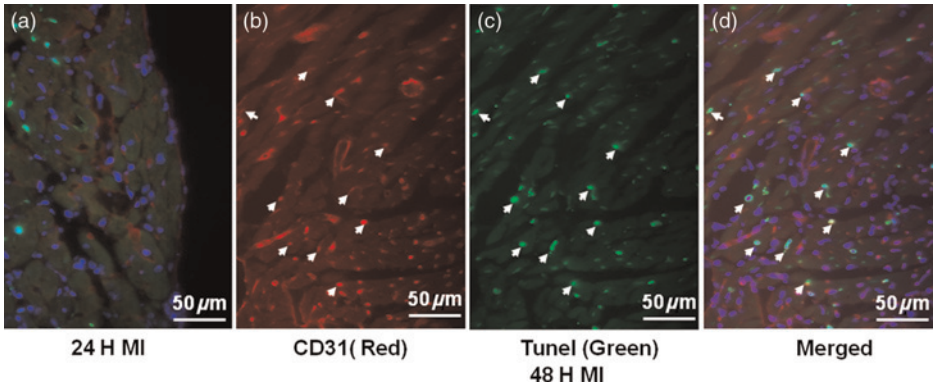

Figure 8 Immunostaining for detection of capillary necrosis. Representative double immunostaining is shown for TUNEL and CD31 in sections of LVs from C57BL6 mice subjected to ischemia for 24 h (a) and 48 h (b–d). The endothelial surface marker CD31-positive cells are stained red (b) and necrotic nuclei are stained green (c). The merged image for 24 h is shown in (a) and for 48 h in (d). Numerous necrotic endothelial cells are seen in the infarct zone (arrows)

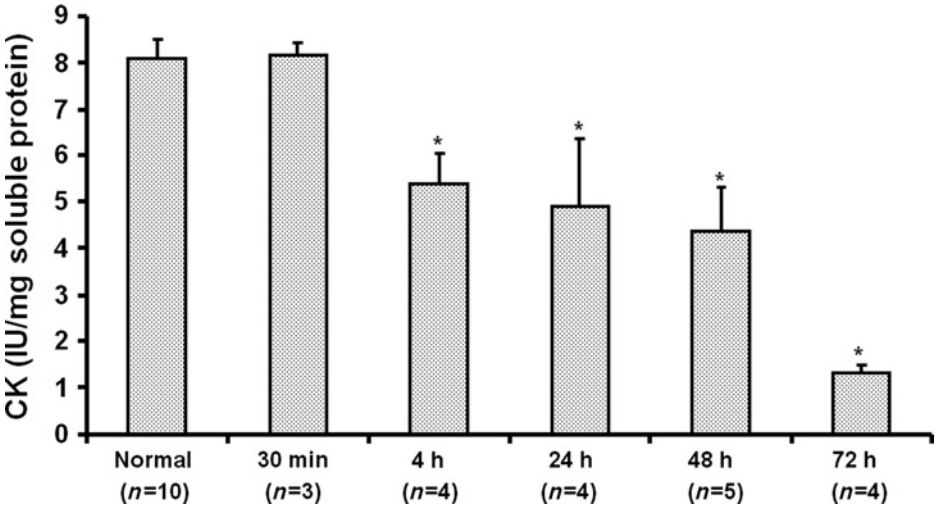

Figure 9 The time course of cardiomyocyte cell death reflected by loss of left ventricular CK activity after MI. Results are means ± SEM; *significantly different from results in normal C57BL6 mice with no ischemia ($P < 0.05$)

The no-reflow phenomenon is manifested by a reduction in myocardial reperfusion distal to an obstruction rectified by re-canalization.[10] Several hypotheses have been advanced to explain no-reflow: distal embolization, ischemic injury, reperfusion injury and susceptibility of coronary microcirculation to injury.[9] There is a dearth of evidence regarding the integrity of vessels in the infarct zone and its loss playing a role in no-reflow.[20] In a case report, histopathological findings from a patient with suspected no-reflow case following coronary intervention for ACS demonstrated necrosis of the vascular wall in small arteries and detachment of endothelial cells.[20] This appeared to be secondary to the microthrombi and plugging of arterioles. We observed the loss of small arterioles and capillaries in the infarct zone within 48 h of ischemia. It is possible that early reperfusion with or without adjunctive vascular protective agents can reduce or preclude vascular rhexis. Thus, diminution of vascular rhexis is an attractive therapeutic target.

Our data demonstrate that vascular cells tolerate an ischemic insult of a longer duration than do myocytes as demonstrated by the time course of CK depletion compared with the time course of vascular rhexis. Cardiomyocyte death is seen as early as four hours after coronary occlusion. By contrast, vascular rhexis becomes evident within 48 h after coronary occlusion. The disparity is consistent with the high oxygen requirements of cardiomyocytes and the proximity of vascular components to intraluminal blood. We observed that hemorrhage in C57BL6 mice is maximal 96 h after coronary occlusion despite the earlier onset of vascular rhexis. The compensatory mechanisms constraining hemorrhage that occur in normal mice are likely to be responsible for the delay in the appearance of hemorrhage. We observed the loss of small- and medium-sized vessels in the infarct zone within 48 h after the onset of ischemia, although many capillaries remained viable at this time. The disparity is likely to be a result of the smaller diffusion distances and lower pressures in capillaries compared with arterioles. Preservation of the coronary microvasculature may be possible and beneficial even when preservation of cardiomyocytes is no longer possible.

It was our initial observation of gross hemorrhage in PKO mice that led us to hypothesize that vascular rhexis occurs beginning within 48 h after MI. The increased fibrinolysis in these mice was responsible for a large amount of hemorrhage seen after MI. When the functional consequences of the fibrinolytic system deficiency were corrected by administration of aprotinin, there was still marked albeit less hemorrhage in the infarct zone. When the PKO mice subjected to infarction were treated with cyclophosphamide to reduce inflammation, hemorrhage was reduced as well. Thus, inflammation may be a contributor to vascular rhexis. Consistent with a recent report, our results demonstrate that PAI-I may be protective in mice by reducing inflammation, maintaining vascular integrity and subsequently reducing fibrosis.[21]

Vascular rhexis may be favorably modified by interventions other than anti-inflammatory ones that may protect the vasculature. Stem cells and VEGF have shown to promote vascular integrity and may prove to reduce vascular rhexis.[22,23] Other promising approaches include the potential use of granulocyte colony-stimulating factor and hypothermia. Both exert cardioprotective effects that may be attributable in part to reduction of vascular rhexis.[24–27]

Loss of integrity of coronary microvasculature, i.e. vascular rhexis, occurs in mice subjected to 48 h of persistent ischemia. This phenomenon may contribute to no-reflow typically seen after macroscopic coronary arterial re-canalization. It may contribute also to negative LV remodeling late after MI.

Author contributions: All authors participated in the design and interpretation of the studies, analysis of the data and review of the manuscript. CJF and AKMTZ contributed equally and are co-first authors; both conducted the experiments. The manuscript was written by CJF, AKMTZ and BES.

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