Mouse model of myocardial remodelling after ischemia: role of intercellular adhesion molecule-1

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Abstract

Objective: We studied the effects of temporary myocardial ischemia and reperfusion on myocyte injury and ventricular remodelling in wildtype and intercellular adhesion molecule-1- (ICAM-1) deficient mice.

Methods: ICAM-1\textsuperscript{2/2} and ICAM-1\textsuperscript{1/1} mice were subjected to 30 min of myocardial ischemia and subsequent reperfusion for 2 h, 1 week and 3 weeks, respectively. The evaluation of tissue damage and scar size was performed with histological sections stained with hematoxilin and eosin. Serum levels of troponin T, creatine kinase and lactate dehydrogenase isoenzyme 1 were evaluated as an index of cardiac cellular damage. Immunohistological analysis was employed to determine cell compositions in ischemic regions.

Results: After myocardial ischemia (30 min) and 2 h reperfusion, elevation in serum troponin T, creatine kinase and lactate dehydrogenase isoenzyme 1 were found in both groups, but significantly reduced in ICAM-1\textsuperscript{2/2} mice compared with wildtype mice (P < 0.05). Absence of a functional ICAM-1 gene in ICAM-1\textsuperscript{2/2} mice resulted in a marked reduction of ischemia–reperfusion injury at the early stage. The damage score and size of the infarct area were lower in ICAM-1\textsuperscript{2/2} mice by 30 min of ischemia and 2 h of reperfusion (1.4 ± 0.54 vs. 2.4 ± 0.47, P < 0.05). The percentage of MAC-1-positive cells in the ischemic region and the border zone was also significantly diminished in groups of ICAM-1\textsuperscript{2/2} mice. Surprisingly, the scar size in ventricles in animals 1 or 3 weeks after ischemia was similar between ICAM-1\textsuperscript{2/2} and ICAM-1\textsuperscript{1/1} mice, although the number of infiltrated MAC-1 positive cells in the scar in wildtype mice was higher.

Conclusion: Our results demonstrate that the absence of ICAM-1 expression results in less myocardial damage induced by ischemia–reperfusion at the early stage, but does not influence the size of myocardial infarction and scar formation.

Keywords: Infarction; Ischemia; Reperfusion; Remodelling

1. Introduction

Myocardial ischemia–reperfusion injury is a common event in the clinic; its hallmark is the characteristic chest pain known as angina pectoris. Pathophysiologic manifestations of transient ischemia include impaired ventricular function, which may lead to hypokinesis, akinesis, or dyskinesis [1–3]. If the cause of the ischemia is not removed within 20 min after onset then myocardial irreversible injury (i.e. infarction) may occur followed by scar formation [1–3]. However, the mechanism of the pathogenesis of ischemia–reperfusion injury is not yet fully understood.

Intercellular adhesion molecule-1 (ICAM-1) belongs to the Ig superfamily of cell adhesion molecules, and is widely expressed on hematopoetic and endothelial cells. It is a ligand for MAC-1 and LFA-1 (CD18/CD11a, b) on leukocytes. Its expression is highly upregulated during inflammation on endothelial cells [4,5]. ICAM-1 is required for the transendothelial migration of neutrophils and...
electrophoresed on agarose gel. ICAM-1

DNA was isolated from tail tissues by enzyme digestion glycolysis and followed by reperfusion for 2 h, 1 and 3 weeks in ICAM-1-deficient mice. We found a significant reduction of myocardial damage with 2 h reperfusion in ICAM-1-deficient mice, but no difference in infarct size between ICAM-1-deficient and wildtype mice 1 and 3 weeks after ischemia.

2. Methods

2.1. Mice

ICAM-1-deficient mice of the C57BL/6J strain [12] were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Three genotypes of ICAM-1−/−, +/+ and +/+ mice were identified using The Jackson Laboratory’s PCR protocol with a slight modification. Briefly, genome DNA was isolated from tail tissues by enzyme digestion and precipitation. A 50-ng amount of DNA in a reaction buffer was incubated with 0.4 μM each primer (oIMR017 5′-CTG AGC CAG CTG GAG TCG -3′; oIMR018 5′-GAG CCG CAG AGC AAA AGA ACG -3′; oIMR019 5′-AGG ACA GCA AGG GGG AGG ATT -3′) at the optimal temperature. After reaction, the mixture was electrophoresed on agarose gel. ICAM-1−/− mice have a 150-bp band; ICAM-1+/+ mice, a 178-bp band; and ICAM-1+/− mice have the both bands. The mice were maintained on a light–dark (12/12 h) cycle at 24°C receiving food and water ad libitum. All procedures were performed according to protocols approved by the Institutional Committee for Use and Care of Laboratory Animals.

2.2. Surgical procedures

The procedure used in the present experiment is similar to that described by Michael et al. [13]. Briefly, mice were anaesthetised with phenobarbital (50 mg/kg, i.p.), fixed in the supine position by taping the extremities and the upper jaw. A midline skin incision from the xiphoid process to the submentum was made. After separating the salivary glands, the muscles overlying the trachea were retracted, and a tracheotomy was performed. A polyethylene tube (No. 90) was carefully inserted into the trachea, taped in place to prevent dislodgement, and connected via a loose junction to a Harvard rodent ventilator (model 687; Harvard Apparatus). After ventilation (tidal volume 1.2 ml/min, rate 110 strokes/min) was started by supplementation of 100% oxygen, the chest was opened by a lateral cut along the left side of the sternum. The whole surgical procedure was performed under the microscope (Olympus SZH 10). With an electrocautery, intercostal blood vessels were coagulated. The chest walls were then retracted by use of 6-0 silk suture for better visualisation of the heart. After removing the pericardial sac and slightly retracting the left auricle, the left descending artery became clearly visible. A 1-mm section of polyethylene-10 tubing was placed on top of the left descending artery to secure the ligation of the left descending artery without damaging the artery. The left descending artery was ligated with an 8-0 silk suture and was evident by discoloration of the left ventricle. The retraction sutures were removed, the chest wall was approximated, and the chest was covered with a small moist swab. After occlusion of 30 min, reperfusion was allowed by cutting the knot on the polyethylene-10 tube.

To test the efficiency of ischemia–reperfusion, Evans blue dye was infused into the common coronary artery via aorta after ligation of the left descending artery or after removing ligation. Fig. 1 shows data of representative animals, indicating efficiency of ischemia and reperfusion. Wildtype (n = 33) and ICAM-1−/− (n = 32) male mice were subjected to 30 min of left descending artery occlusion and followed by reperfusion for 2 h, 1 week and 3 weeks, respectively. The time points of 1 and 3 weeks for studying the effects of ICAM-1 on myocardial remodelling were chosen based on our preliminary results because the strongest inflammatory response was observed 1 week after ischemia, and maturated scar in the infarct area was formed at 3 weeks.

2.3. Determination of serum troponin T, creatine kinase and lactate dehydrogenase isoenzyme 1

From each mouse, heparin-blood was collected when they were sacrificed. Blood concentrations of troponin T were measured as an index of cardiac cellular damage by using the quantitative rapid assay kit (Roche Diagnostics) [14]. A 10-μl volume of plasma was used, and each test strip was rechecked visually.

Creatine kinase and lactate dehydrogenase isoenzyme 1 (LDH-1) were electrophoretically analysed using commercially available kits (Paragon, Fullerton, CA, USA). The gels were scanned with a densitometer and the relative activities of each isoenzyme fraction was calculated against total enzyme activities.

2.4. Tissue preparation and damage evaluation

After reperfusion, the heart was harvested and cut into
two portions 1 mm below the ligation suture. The upper portion was frozen in liquid nitrogen for preparation of frozen sections. The lower portion was fixed in 4% formaldehyde at 4°C overnight and embedded in paraffin [15]. Sections (5-μm thick) were cut from the cross area and stained with hematoxilin and eosin (HE) for histological evaluation of tissue damage. For semi-quantitative estimation of tissue damage, the methods described by Zingarelli et al. [16] were used. According to this score system the following criteria were used: score 0, no damage; score 1 (mild), interstitial edema and focal necrosis; score 2 (moderate), diffuse myocardial cell swelling and necrosis; score 3 (severe), necrosis with the presence of contraction bands and neutrophil infiltrate; and score 4 (highly severe), widespread necrosis with the presence of contraction bands, leukocyte infiltrate, and haemorrhage. Six animals in each group were included, and ten sections from each animal were evaluated.

2.5. Analysis of the infarct or scar size

For infarct or scar area measurement, sections were reviewed using a B×60 microscope (Zeiss, Jena, Germany) equipped with a Sony 3CCD camera and television monitor. The scar was defined as the region between the living myocytes and cardiac membrane. Using a transmission scanning microscope (Bio-Rad), equipped with a 488-nm argon ion laser and Plan Neofluar 10×/0.3 oculars and connected the program START LSM 510, images were scanned and saved. Areas were measured and recorded in square micrometers. In the statistical analyses, the individual values for the area from each animal at each time point were averaged. The investigator for this analysis was blinded to group assignment.

2.6. Immunohistochemical analysis

Serial 5 μm-thick frozen sections were cut from cryopreserved tissue blocks, fixed in a cold acetone–chloroform (1:1) mixture for 10 min, and washed with phosphate-buffered saline (PBS) for 20 min. The sections were subsequently placed in a humidified chamber, where they were overlaid with a rat monoclonal antibody against mouse MAC-1-positive leukocytes and incubated for 1 h at room temperature [15]. After washing with PBS, sections were incubated with rabbit-anti-rat Ig for 1 h. Sections
were washed with PBS three times, incubated with rat monoclonal antibody alkaline phosphatase-anti-alkaline phosphatase (Dakopatts) for 30 min, and developed for 20 min at room temperature on a shaker using a substrate solution (Sigma, St. Louis, MO, USA). MAC-1 positive cells were counted at 10×40 magnification.

2.7. Statistical analysis

Statistical analysis was performed on a Macintosh computer with STATVIEW SE+GRAPHICS software (version 1991) using the Mann–Withney U test. Results were given as means±standard deviations (S.D.). A P value of <0.05 was considered to be significant.

3. Results

From the total of 65 animals which were subjected to surgery, 52 (80%) survived and were included in the experiments. Mortality was highest within the first hours of surgery because of severe bleeding from intercostal vessels. These animals were excluded from the study. Between ICAM-1−/− and ICAM-1+/+ mice there was no significant difference in mortality (six vs. seven mice).

3.1. Elevated troponin T, creatine kinase and LDH-1 levels in serum

It has been shown that cardiac troponin T is a sensitive, specific biomarker of cardiac injury in laboratory animals, including mice [17]. Recently, we demonstrated that troponin T was more cardiospecific than creatine kinase or lactate dehydrogenase isozyme activities in mice (Metzler et al., unpublished observations). In wildtype mice, 30 min of occlusion of the left descending artery followed by 2 h reperfusion resulted in marked myocardial damage. Serum troponin T, as an index of myocyte damage, increased to 81 ng/ml over a control below the level of quantification. Absence of a functional ICAM-1 gene in ICAM-1−/− mice resulted in a significant reduction of troponin T by 40% compared with the wildtype mice (50.1±10 vs. 81±19, P<0.05; Fig. 2). As expected, serum troponin T was not detectable 1 week after myocardial ischemia. Similarly, serum creatine kinase and LDH-1 activities increased markedly in the ischemia–reperfusion group, and were significantly lower in ICAM-1−/− mice (Fig. 2).

3.2. Acute myocardial damage in response to ischemia–reperfusion

Histologically, normal hearts from sham operated wildtype and ICAM-1-deficient mice revealed a similar characteristic (Fig. 3A and B). An ischemic period of 30 min due to the occlusion of the left descending artery followed by 2 h reperfusion resulted in a marked myocardial necrosis.
Fig. 3. Histological evaluation of myocardial damage after ischemia–reperfusion. The left descending artery was occluded for 30 min and reperfused for 2 h. The heart was harvested, fixed with 4% formaldehyde and embedded in paraffin. Sections were stained with hematoxilin and eosin. The myocardial damage was evaluated and scored according to the method described by Zingarelli et al. [16]. (A and B) Sections from sham-operated hearts; (C and D) hearts of ischemia–reperfusion. A–D are representative photographs. Note that typically damaged tissues were marked in (D). (E) Statistical data of means±S.D. (n=6). *, Significant difference from wildtype mice, P<0.05.

Fig. 4. Immunohistological evaluation of MAC-1-positive cells in the ischemic region. Sham-operated hearts and hearts with ischemia–reperfusion (30 min–2 h) were harvested and frozen sections were prepared for immunohistochemical staining. Sections were incubated with a rat monoclonal antibody against MAC-1-positive leukocytes and visualised with alkaline phosphatase–anti-alkaline phosphatase system. Sections were counterstained with hematoxilin. Positively stained cells and total nuclei in the ischemic regions were enumerated under the microscope. Graph shows statistical data of means±S.D. *, Significant difference from wildtype mice, P<0.05.
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(4.6±1.4 vs. 13.4±4.2%, P<0.05; Fig. 4). MAC-1 positive cells were also observed in the border zone.

3.3. Myocardial remodelling in response to ischemia

Clearly, ICAM-1 mediates cardioprotection in the early stage of ischemia–reperfusion injury, but it is not known whether such protection is true for cardiac remodelling or scar formation after ischemia. To study this issue, we established and characterised a mouse model of myocardial remodelling after 30 min of ischemia. Data shown in Fig. 5 indicate histological features of scars reperfused for 1 week after 30 min of ischemia. Myocardial ischemia resulted in myocyte death followed by scar formation in both ICAM-1−/− and ICAM-1+/+ mice. When scar areas were quantified using a laser scanning microscope, no significant difference in scar size between ICAM-1−/− and ICAM-1+/+ mice was found (5.5×10^5±0.064 vs. 5.6×10^5±0.10; Fig. 5). Cell density in the scar area of wildtype mice was much higher than that of ICAM-1-deficient mice (data not shown). Immunohistochemical analysis demonstrated the presence of MAC-1-positive leukocytes in the infarct areas of both groups. Semi-quantitative data indicate that the number of MAC-1-positive leukocytes infiltrated in infarct areas in ICAM-1−/− mice was significantly lower compared to the wildtype mice (Fig. 6). Thus, the absence of ICAM-1 resulted in fewer cells infiltrated into infarct tissues, which, however, did not influence infarct size.

To further investigate the effect of ICAM-1 on myocardial remodelling, we performed experiments with reperfu-

Fig. 5. Evaluation of scar size in hearts 1 week after ischemia. The left descending artery was occluded for 30 min and reperfused for 1 week. The heart was harvested, fixed with 4% formaldehyde and embedded in paraffin. Sections were stained with hematoxilin and eosin. The scar area was measured using a scanning microscope as described in the Methods. Graph shows statistical data from ICAM-1−/− and ICAM-1+/+ mice. Arrows indicate the border between scar and myocytes.

Fig. 6. Immunohistological staining for MAC-1-positive cells in the scar region. Frozen sections were prepared from hearts of ICAM-1−/− (A) and ICAM-1+/+ (B) mice treated with ischemia for 30 min and reperfusion for 1 week. Sections were incubated with a rat monoclonal antibody against MAC-1-positive leukocytes and visualised with alkaline phosphatase–anti-alkaline phosphatase system. Sections were counterstained with hematoxilin. Positively stained cells and total nuclei in the ischemic regions were enumerated using a scanning microscope. Arrows indicate examples of positive stained cells. Panel C shows statistical data of means±S.D. *, Significant difference between two groups, P<0.05.

4. Discussion

In the present study, the mouse model of ischemia–reperfusion can be used to study not only acute reperfusion injury, but also the subsequent remodelling of myocardium. We demonstrate that it is possible to occlude the left coronary artery for 30 min and then allow the mice to live for a longer period of time. Temporary coronary ischemia with subsequent reperfusion in the mouse is similar to the clinical setting seen in humans with myocardial infarction followed by thrombolytic therapy or cardiac catheterization. Our model differs from the model of a permanent LAD occlusion, i.e. lacking aneurysmal infarcts. Therefore, this animal model of long-term reperfusion and
remodelling which occurs after myocardial infarction could be useful for studying the pathogenesis of ischemia–reperfusion injury as well as myocardial remodelling. Experimental studies have shown that inflammatory tissue damage due to ischemia–reperfusion is caused mostly by polymorphonuclear leukocytes [18]. Leukocyte adherence to endothelial cells and the transmigration involve several adhesion molecules on the surfaces on both cell types. ICAM-1 which plays an important role in that context is constitutively expressed at low levels on endothelial cells, and has been shown to be upregulated in ischemic and reperfused myocardium and also in the viable border zone of ischemic myocardium in humans and animals within a few hours [19–21]. There is an abundance of data suggesting an involvement of ICAM-1–CD18 interactions in the recruitment of circulating leukocytes into the myocardium and also in the development of subsequent myocardial cell damage after ischemia and reperfusion [22,23]. Our data demonstrate significant decreases in numbers of MAC-1-positive leukocytes in the infarcted region and also in the border zone of ischemic heart of ICAM-1\(^{-/-}\) mice, although the number of circulating leukocytes has been shown to be similar in ICAM-1\(^{-/-}\) and wildtype mice [11]. Recently, Lu et al. [24] demonstrated that LFA-1 reduced neutrophil migration by 78% and MAC-1- disruption decreased neutrophil degranulation. Our results are consistent with this finding because ICAM-1 is the ligand for both LFA-1 and MAC-1 (CD18/CD11a/b) on leukocytes. Therefore, a decreased number of MAC-1 positive leukocytes in the infarcted region of ICAM-1\(^{-/-}\) mice may be responsible for the reduction of myocardial infarction size 2 h after reperfusion, in which release of cytotoxic agents from the
leukocytes, such as free radicals, can lead to cardiomyocyte damage [25,26].

Postinfarct-remodelling is of significant clinical interest because the changes usually result in an enlargement of the ventricle with the development of heart failure. In principle, myocardial remodelling after myocardial infarction can be divided into two major steps [1–3]. First, the proliferation of myofibroblasts with synthesis of extracellular-matrix proteins such as collagen and fibrinogen. During this process, the continuous recruitment of leukocytes by ICAM-1 and the subsequent release of proinflammatory cytokines such as interleukin-1 may play a pivotal role. Second phase is characterised by angiogenesis following a myocardial infarction. Although no data addressing the role of ICAM-1 in the angiogenesis of the heart in response to ischemia–reperfusion exists so far, Becker et al. [27] hypothesised that ICAM-1 mediated-leukocyte adhesion is a key event in early (corneal) angiogenesis. In the current study, we demonstrate that the numbers of infiltrated leukocytes in the infarct area is negatively associated with scar size after ischemic injury during the late phase, and we provide the first evidence that ICAM-1 has no protective role in the myocardial remodelling at the late stage. Other reports demonstrated that leukocyte influx into the previously infarcted area may be beneficial for tissue repair by enhancing phagocytosis as well as by producing growth factors and cytokines that may modulate scar formation [28–30]. Thus, we postulate that ICAM-1-mediated leukocyte infiltration in ischemic tissues might be a double-edged sword that could result in myocyte damage at the early stage of reperfusion and that could help promote myocyte repairing at the late stage.

In summary, our findings clearly demonstrate that ICAM-1 plays an important role in the early stages of cardiac damage following ischemia–reperfusion injury. In this process, ICAM-1-mediated leukocyte adhesion and subsequent infiltration into the infarct area could be responsible for myocyte damage via released free radicals. If leukocyte recruitment can be blocked within 2 h of ischemia, the inflammatory process should be less serious and a reduction of myocardial damage may be achieved. After that point, leukocyte infiltration into the infarcted area may help promote repairing of the cardiac tissue, and consequently, the enhancement of leukocyte recruitment might be beneficial. Thus, our data provide basic information concerning the role of ICAM-1 in ischemia–reperfusion injury as well as cardiac remodelling, which could be helpful for the treatment of ischemia–reperfusion injury in humans.

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