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SUPPLEMENTARY DATA

METHODS

Mouse myocardial infarction model

Briefly, before any surgical procedure, intraperitoneal buprenorphine (0.1 mg/kg) and meloxicam (0.3 mg/kg) were administered and then mice were anesthetized by inhalation of 5% isoflurane (Abbott Laboratories, Chicago, IL, United States) delivered in 100% oxygen medical grade air in an anesthetic chamber. The mice were then placed on the surgical board and underwent tracheal intubation connected to a rodent ventilator (Minivent type 845, Panlab Harvard Apparatus, Barcelona, Spain) set at a tidal volume of 200 µL, and a rate of 110 breaths per minute supplemented with 100% oxygen and isoflurane (2%) at a flow rate of 0.2 L/min. The mice were maintained at a constant temperature of 37ºC with a heating pad. During the experiments, the electrocardiogram and animal temperature via a rectal probe were continuously monitored (Mouse Monitor S, Indus Instruments, Webster, TX, United States). After opening the thorax, a left minithoracotomy was performed at the height of the fourth intercostal space. Then part of the pericardium was removed and the coronary artery was located with a microscope for surgery. The occlusion process was carried out using a needle holder for microsurgery and 6-0 monofilament suture thread. The occlusion point was passed approximately 1-2 mm from the apex of the left atrium when in its normal position.

Drug administration protocol

Intraperitoneal buprenorphine (0.05 mg/kg, twice daily) and meloxicam (0.3 mg/kg, once daily) was administered for 5 days after surgery. As previously reported, vascular endothelial growth factor (VEGF)-A_{165}b blocking antibody (5 µg/mL, cat# MAB3045, R&D Systems, Minneapolis, MN) was administered ip 1 hour after myocardial infarction (MI) induction and on postoperative days 1, 3, 7, and 14.[1] As control, nonspecific mouse IgG was administered in a similar manner.

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Positron emission tomography/computed tomography

One day prior to sacrifice, mice were starved for 8 hours before ^{18}F-2-fluor-2-deoxiglucose (^{18}F-FDG; Curium Pharma, Madrid, Spain) injection. Mice were anesthetized with isoflurane (2% in 100% oxygen; Abbott Laboratories, Chicago, IL, United States) and afterwards ^{18}F-FDG (0.2-0.3 mCi in 0.2 mL) was injected ip. The administered dose (FDG activity) was corrected for body weight. Sixty minutes after ^{18}F-FDG injection, anesthetized animals were placed in a supine body position and 20 minutes of static positron emission tomography (PET) images and 7 minutes of computed tomography (CT) images were acquired. The PET images were obtained with the Albira small animal PET (Bruker Biospin GmbH, Rheinstetten, Germany). ^{18}F-FDG biodistribution in the heart was compared between all experimental groups. Regions of interest/volume of interest (kBq/cm^3) were manually drawn over the heart with AMIDE software.

Echocardiography

Prior to sacrifice, mice were anesthetized with isoflurane and a transthoracic echocardiogram of the left ventricle (LV) was obtained using a 50-MHz linear array scan-head interfaced with Vivid S5 equipment (GE Healthcare, Chicago, IL, United States). Mice were placed on a heating pad under light anesthesia with isoflurane and measurements were taken by a blinded operator. From the M-mode images, LV end-diastolic and end-systolic diameter, fractional shortening, and ejection fraction were calculated.

Blood sampling

Twenty-one days after MI induction, animals were reanesthetized, placed in a supine position, and a blood sample was drawn from the cava vein. Next, the anesthetized mice were sacrificed by cervical dislocation. Blood was centrifuged at 2500 rpm for 15 min, and serum was immediately stored at −80ºC until further analyses were performed.

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Infarct size analysis

After sacrifice, hearts were removed and cut into 1-mm thick short-axis slices. First, each slide was viewed under room light and photographed. Second, slices were incubated in 2% 2,3,5-triphenyltetrazolium chloride (Sigma Aldrich, San Luis, MO) solution at 37ºC for 20 minutes. Subsequently they were viewed under room light and photographed.

Myocardial slices were fixed in 4% paraformaldehyde acid, embedded in paraffin, sectioned (5 µm), and mounted on double gelatin-coated glass slides. To further characterize mice myocardium, heart samples were stained with hematoxylin-eosin (Sigma Aldrich, San Luis, MO) for histological analysis and infarct size quantification and with Masson's trichromic staining (PanReac AppliChem, Chicago, IL) to detect the presence of fibrosis.

After digitizing the images, manual offline quantification of all microscopic images was performed in a dedicated laboratory by a trained observer unaware of the experimental protocol applied. All numerical data analyses were performed using Image-Pro Plus analysis software (Media Cybernetics Inc, Rockville, MD).

VEGF-A_{165}b detection

Mouse serum levels of VEGF-A_{165}b were measured using commercially available ELISA kit (cat# MBS9357293 MyBiosource Inc, San Diego, CA), according to the manufacturer's recommendations.

VEGF-A_{165}b detection and neoangiogenesis quantification in myocardial tissue samples

For neovascularization detection, the dehydrated sections were treated with proteinase K (cat#s3020, Dako, Agilent Technologies, Santa Clara, CA) for 30 minutes at room temperature for antigen retrieval. After incubation with peroxidase blocking solution (cat# s3023, Dako, Agilent Technologies, Santa Clara, CA) and blockage with goat serum (15%) (cat# ab7481, Abcam, Cambridge, UK), samples were incubated overnight (4ºC) with the antibody rabbit polyclonal antimouse-CD31 (1:50 dilution, cat# ab28364, Abcam) diluted in Antibody Diluent (cat# s2022, Dako, Agilent Technologies, Santa Clara, CA, United States).

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Specific labelling was detected with a labelled polymer-HRP antirabbit secondary antibody and revealed with 3,3'-diaminobenzidine (cat# 4010, Dako, Agilent Technologies, Santa Clara, CA).

For VEGF-A_{165}b detection, double immunofluorescence analysis was performed, the samples were incubated overnight (4ºC) with mouse anti-VEGF-A_{165}b antibody, clone 56/1 (1:50 dilution, cat# MABC595, Sigma Aldrich, San Luis, MO) and rabbit polyclonal antimouse-CD31 (1:50 dilution, cat# ab28364, Abcam). Specific labelling was detected with an Alexa Fluor 488 goat antimouse secondary antibody (1:1000 dilution, cat# A11001, ThermoFisher Scientific, MA) or an Alexa Fluor 594 goat antirabbit secondary antibody (1:1000 dilution, cat# A11037, ThermoFisher Scientific, MA). Nuclei were stained with DAPI. To confirm thee specificity of antibodies, isotype controls (cat# 172730, Abcam, Cambridge, UK) or secondary antibodies only were used as negative controls. Five independent fields from each infarct or control sections were captured (Axio Observer A1, Carl Zeiss, Oberkochen, Germany), digitized and then analyzed with Image-Pro Plus analysis software (Media Cybernetics Inc, Rockville, MD). Scoring was performed blinded on coded slides.

Western blotting

After sacrifice, infarct myocardial samples were lysed, and protein concentration was determined using the Bradford method.[2] Samples were denatured, subjected to SDS-PAGE using a 10% running gel, and transferred to a nitrocellulose membrane. Nonspecific binding sites were blocked with 3% bovine serum albumin in TBS solution and membranes were incubated overnight with rabbit polyclonal antibodies against mouse phospho-AKT (Ser473) (1:200 dilution, cat# 4085, Cell Signaling Technology, Danvers, MA), AKT (1:200 dilution, cat#4685, Cell Signaling Technology, Danvers, MA), CD31 (1:500, cat# 28364, Abcam, Cambridge, UK), and GAPDH (1:2000 cat# G8795, ThermoFisher Scientific, MA). Membranes were subsequently washed, incubated for 1 additional hour with the secondary HRP-linked antirabbit antibody (1:2000 dilution, cat#0448, Dako, Glostrup, Denmark) and developed using an ECL procedure (GE Healthcare, Madrid, Spain). Signals were recorded using a luminescent analyser (FujiFilm Image Reader LAS 4000, Fuji, Tokyo, Japan).

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Interleukin (IL)-6 detection

Mouse serum levels of IL-6 were measured using a commercially available ELISA kit (cat# M6000B Quantikine, R&D Systems, Minneapolis, MN), according to the manufacturer's recommendations.

Study in ST-segment elevation myocardial infarction patients

Baseline characteristics and blood samples

Baseline characteristics were prospectively registered in all cases. Thrombolysis in Myocardial Infarction flow grade in the culprit artery (before and after reperfusion) was analyzed. Patients were managed both in-hospital and after discharge by a specific ST-segment elevation myocardial infarction (STEMI) unit, and current recommendations were strictly followed.[3] Further details on patients' characteristics are shown in table 2 of the supplementary data.

Blood samples were isolated 24 hours after coronary revascularization, centrifuged at 2300 rpm for 15 minutes and serum was immediately stored at −80 ºC until further analyses were performed.

Cardiovascular magnetic resonance

Cardiovascular magnetic resonance (CMR) (1.5 T unit, Magnetom Sonata; Siemens, Erlangen, Germany) was performed 182 ± 40 days (6-month CMR) after STEMI, in accordance with our laboratory protocol and current recommendations.[4,5] All studies were performed and analyzed by 2 cardiologists specialized in CMR with 15 years of experience using customized software (QMASS MR 6.1.5, Medis, Leiden, The Netherlands). CMR data were prospectively recorded and immediately included in the database.

Images were acquired by a phased-array body surface coil during breath-holds and were triggered by electrocardiography. Cine images were acquired in 2-, 3-, and 4-chamber views, and in short-axis views using a steady-state free precession sequence (repetition time/echo time: 2.8/1.2 ms; flip angle: 58 degrees; matrix: 256 × 300; field of view: 320 × 270 mm; slice thickness: 7 mm).[4,5]

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Late gadolinium enhancement imaging was performed 10 to 15 minutes after administration of 0.1 mmol/kg of gadolinium diethylenetriaminepentaacetic acid (Magnograf, Juste S.A.Q.F., Madrid, Spain) in the same locations as in the cine images using a segmented inversion recovery steady-state free precession sequence (repetition time/echo time: 750/1.26 ms; flip angle: 45 degrees; matrix: 256 × 184; field of view: 340 × 235 mm; slice thickness: 7 mm). Inversion time was adjusted to nullify normal myocardium.[4,5]

Black blood, T_2-weighted short T_I inversion recovery sequences in the same short-axis view as the cine sequences, all in mid-diastole, were carried out. A half-Fourier acquisition single-shot turbo-spin echo multisection sequence was used (recovery time: 2 R-R intervals; echo time: 33 ms; inversion time: 170 ms; slice thickness: 8 mm; interslice interval: 2 mm; flip angle: 160 degrees; matrix: 256 × 151; bandwidth: 781 Hz/pixel). Additionally, a segmented turbo-spin echo sequence was obtained with 1 slice per breath-hold (recovery time: 2 R-R intervals; echo time: 100 ms; inversion time: 170 ms; slice thickness: 8 mm; interslice interval: 2 mm; flip angle: 180 degrees; matrix: 256 × 146; bandwidth: 235 Hz/pixel).[4,5]

The interobserver variability for the calculation of traditional CMR indexes used in the present study in our laboratory has been previously reported and is less than 5%.[5]

LV ejection fraction (%) represents the most robust CMR index for predicting patient outcomes[6] and was calculated by manual planimetry of endocardial and epicardial borders in short-axis view cine images. Cutoff value for LV ejection fraction dichotomization (depressed vs preserved) was based on previously validated data.[4,5]

Follow-Up

Major adverse cardiac events consisted of cardiac death, admission for nonfatal reinfarction, or for heart failure, whichever occurred first. Current definitions were applied.[3,7] Major adverse cardiac events were systematically reviewed from the medical history of each patient available on the hospital database and consensus between 2 cardiologists was required to finally classify a cardiac event.

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VEGF-A_{165}b detection

Human serum levels of VEGF-A_{165}b were measured using commercially available ELISA kit (cat #MBS109074, My Biosource Inc, San Diego, CA), according to the manufacturer's recommendations.

REFERENCES

1. Kikuchi R, Nakamura K, MacLauchlan S, et al. An antiangiogenic isoform of VEGF-A contributes to impaired vascularization in peripheral artery disease. *Nat Med.* 2014;20:1464-1471.

2. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1978;72:248-254.

3. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD. Third universal definition of myocardial infarction. *Eur Heart J.* 2012;33:2551-2567.

4. Bodi V, Monmeneu JV, Ortiz-Perez JT, et al. Prediction of reverse remodeling at cardiac MR imaging soon after first ST-segment-elevation myocardial infarction: Results of a large prospective registry. *Radiology.* 2016;278:54-63.

5. Gavara J, Rodriguez-Palomares JF, Valente F, et al. Prognostic value of strain by tissue tracking cardiac magnetic resonance after ST-segment elevation myocardial infarction. *JACC Cardiovasc Imaging.* 2018;11:1448-1457.

6. Ibañez B, Aletras AH, Arai AE, et al. Cardiac MRI endopoints in myocardial infarction experimental and clinical trials. *J Am Coll Cardiol.* 2019;75:238-256.

7. Ponikowski P, Voors AA, Anker SD, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). *Eur Heart J.* 2016;37:2129-2200.

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Table 1 of the supplementary data

Comparison of baseline characteristics between STEMI patients and controls.

	STEMI n = 104	Controls n = 25	*P*
Age, y	60 ± 13	64 ± 12	.16
Male sex, %	79 (76)	18 (72)	.52
Diabetes mellitus, %	27 (26)	7 (28)	.75
Hypertension, %	60 (58)	15 (60)	.77
Hypercholesterolemia, %	47 (45)	11 (44)	.89
Smoker, %	59 (57)	7 (28)	<.01

STEMI, ST-segment elevation myocardial infarction.

Data are expressed as No. (%) or mean ± standard deviation.

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Table 2 of the supplementary data

Baseline, electrocardiographic, laboratory, and angiographic characteristics of all STEMI patients.

	All patients n = 104
Age, y	60 ± 13
Male sex, %	79 (76)
Diabetes mellitus, %	27 (26)
Hypertension, %	60 (58)
Hypercholesterolemia, %	47 (45)
Smoker, %	59 (57)
Heart rate, beats per minute	73 ± 16
Systolic pressure, mmHg	134 ± 30
Time to reperfusion, min ST-segment resolution, %	187 [139–393] 76 ± 25
Anterior infarction, %	54 (52)
Multivessel disease, %	31 (30)

STEMI, ST-segment elevation myocardial infarction.

Time to reperfusion is expressed as median [percentile 25–percentile 75].

Data are expressed as No. (%) or mean ± standard deviation

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SUPPLEMENTARY FIGURES

Figure 1 of the supplementary data. Schematic diagram showing experimental groups, treatment, and tests conducted in mice. MI, myocardial infarction; PET/CT, positron emission tomography/computed tomography; VEGF, vascular endothelial growth factor.

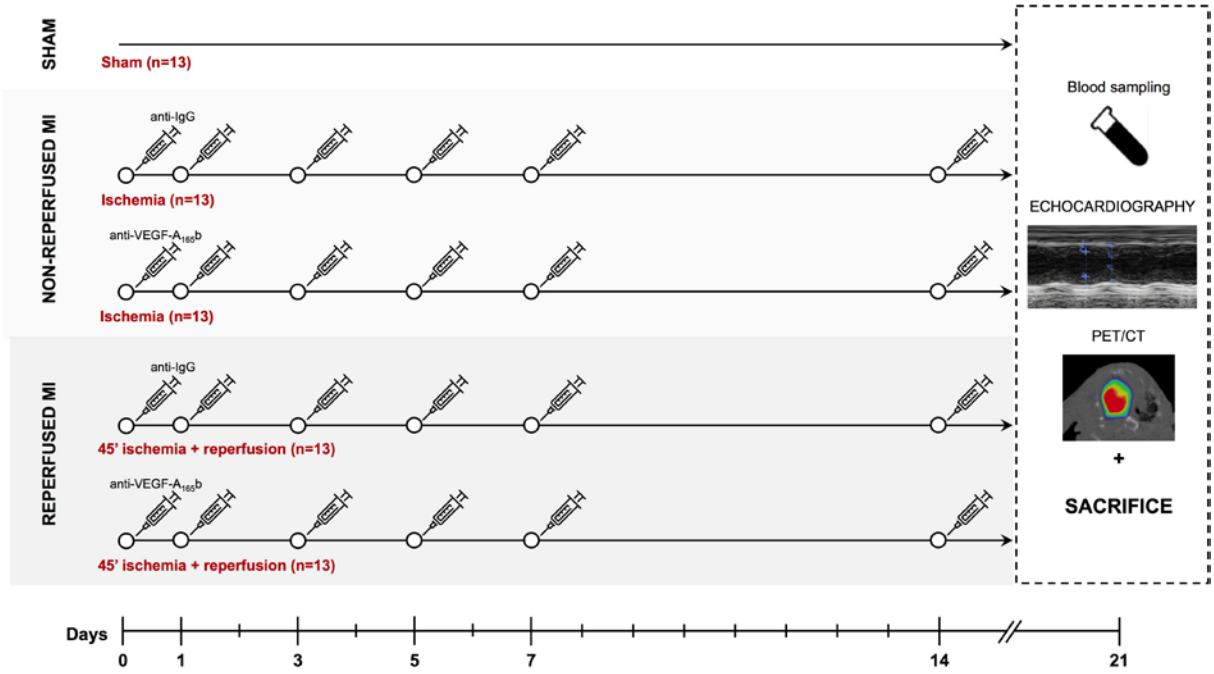

Figure 2 of the supplementary data. Flowchart showing the enrolment protocol of STEMI patients. CMR, cardiovascular magnetic resonance; STEMI, ST-segment elevation myocardial infarction.