Kuhlmann et al. www.jem.org/cgi/doi/10.1084/jem.20051151

SUPPLEMENTAL MATERIALS AND METHODS

Perioperative anesthesia.

For all surgical interventions, combination anesthesia was used composed of 0.05 mg/ml 10.67% fentanyl (Janssen-Cilag), 5 mg/ml 5.33% midazolam (Hoffmann-LaRoche AG), and 1 mg/ml 1.33% medetomidin (Pfizer) applied i.p. at a dose of 15 ml/kg. After surgery, anesthesia was antagonized by a combination of 0.4 mg/ml 0.67% naloxone (Curamed Pharma), 5 mg/ml 1.33% atipamezole (Pfizer), and 0.1 mg/ml 6.67% flumazenil (Hoffmann-La Roche) applied i.p. at a dose of 15 ml/kg. Postoperative analgesics were given as needed.

Induction of myocardial infarction.

Animals were anesthetized, fixed on a heater mat, and ventilated by a rodent respirator (type 845; Hugo Sachs Elektronik/Harvard Apparatus). After left thoracotomy in the fourth intercostal space and transection of the pericardium, the left anterior descending coronary artery was ligated proximal to the first diagonal branch. The chest was closed, and standardized postsurgical analgesic treatment was applied. The perioperative mortality was not different between treatment groups (G-CSF/SCF 37 of 67 animals [55.8%], control 23 of 55 [41.8%]; $P > 0.2$).

Echocardiography and surface electrocardiogram (ECG) recordings.

In brief, the heart was visualized in parasternal long and short axes and in apical four-chamber views using a 15-MHz linear transducer and a 12-MHz Doppler transducer (Agilent Technologies Sonos 5500; Philips). Before the echocardiographic examination, a 12-lead ECG was recorded using published criteria on a digital ECG recording system (EMKA technologies; reference 1). Data were stored on digital media for offline analysis.

Left ventricular function was measured as fractional shortening. To assess regional wall motion abnormalities, fractional shortening was determined in M mode recordings at different regions of the left ventricle; i.e., close to the papillary muscles, in the mid-third of the left ventricle, and at the apical left ventricle. Akinetic areas were identified by regional wall motion abnormalities in 2D echocardiographic recordings. Infarct size was estimated in short- and long-axis views using the circumference of akinetic segments and by estimating the area of the akinetic zone. Trans-aortic Doppler flow signals were used to assess stroke volume. Cardiac output was calculated from stroke volume and heart rate.

Immunofluorescence.

Frozen hearts were cut into serial cross sections (10 μm) and processed according to an established protocol (2). In brief, slides were incubated overnight at 4°C with the primary antibody, followed by incubation for 50 min with biotinylated secondary antibodies, which were finally labeled by a streptavidin-coupled fluorescent dye (Alexa Fluor 594 streptavidin; Invitrogen; reference 3), or the immunohistochemical signal was further amplified after incubation with the secondary antibody using the TSA Fluorescence System with Cyanine 3 (Cy3) as the final label (NEL704A; PerkinElmer). For coimmunolocalization of connexin43 and fibroblasts, connexin43-stained sections were incubated overnight at 4°C with monoclonal rat anti-reticular fibroblast antibodies (clone ER-TR7; Novus Biologicals Inc.), followed by incubation for 50 min with fluorescein-conjugated anti–rat antibodies (FI-4001; Vector Laboratories). Nuclear counterstaining was achieved using a fluorescence-saving mounting medium containing DAPI (Vector Laboratories).

Primers used for real-time RT-PCR.

The following primers were used: CSF forward primer: 5′-TTAAGTCCCTGGAGCAAGT-3′; CSF reverse primer: 5′-CAGCAACACCAGCTCCTC-3′; CSF receptor forward primer: 5′-CCCAGCTACAATAACAGCTA-3′; and CSF receptor reverse primer: 5′-GCTGAAAGGGATTTATGTTG-3′.

REFERENCES

1. Fabritz, L., P. Kirchhof, L. Fortmuller, J.A. Auchampach, H.A. Baba, G. Breithardt, J. Neumann, P. Boknik, and W. Schmitz. 2004. Gene dose-dependent atrial arrhythmias, heart block, and brady-cardiomyopathy in mice overexpressing A(3) adenosine receptors. *Cardiovasc. Res.* 62:500–508.
2. Mueller, M., K. Wacker, W.F. Hickey, E.B. Ringelstein, and R. Kiefer. 2000. Co-localization of multiple antigens and specific DNA. A novel method using methyl methacrylate-embedded semithin serial sections and catalyzed reporter deposition. *Am. J. Pathol.* 157:1829–1838.
3. Panchuk-Voloshina, N., R.P. Haugland, J. Bishop-Stewart, M.K. Bhalgat, P.J. Millard, F. Mao, W.Y. Leung, and R.P. Haugland. 1999. Alexa dyes, a series of new fluorescent dyes that yield exceptionally bright, photostable conjugates. *J. Histochem. Cytochem.* 47:1179–1188.