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Original article

Experimental model of myocardial infarction: Histopathology and reperfusion damage revisited☆

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ABSTRACT

The goal of this pilot study was to create an experimental model of myocardial infarction (for subsequent evaluation of the effectiveness of an alternative way of stem cell application – intracoronary cell infusion in the management of acute myocardial infarction).

Four experimental animals, female pigs weighing between 30 and 40 kg, were used in the initial phase of this study to create an experimental model of acute myocardial infarction. An experimental myocardial infarction was performed via occlusion of the interventricular arm of the left coronary artery for 90 min. The hearts were examined 1 h, 3 days, 5 days, and 7 days after the procedure. Macroscopically, red infarction characteristic of reperfusion was found. Microscopically, the healing process with granulation tissue production/collagen deposition was remarkably accelerated compared to literature data.

Repair processes in reperfused experimental myocardial infarction and/or reperfused autopsy specimens should not be evaluated on the basis of literature data only. Large collections of extracellular calcium were present. This phenomenon is not well described in the literature and probably has the potential for significantly interfering with the repair process. The histopathology of reperfused acute myoardial infarction deserves to be studied in further investigations.

Introduction

Animal models are widely used for simulating human diseases and in pathological processes, including acute myocardial infarction (AMI). Regarding AMI, many models are used to examine an attractive concept of regeneration of the infarcted myocardium using stem cell therapy.

Evaluation of infarcted tissue by a histopathologist is usually based on classic knowledge sources [3,6], without addressing issues such as age and species of experimental animals and a possible influence of reperfusion. These issues can interfere with expected kinetics of healing processes and thus possibly alter experimental results.

As part of a larger study (whose aim was to assess the effectiveness of an alternative stem cell transplantation technique – intracoronary stem cell infusion in a higher medium volume without catheter balloon inflations [5]) we made histopathologic observations which differ from those reported in the literature.

Material and methods

Animals

Four experimental animals, female pigs weighing between 30 and 40 kg, were used. The animals were used in accordance with the Guide for the Care and Use of Laboratory Animals (DHHS publ. No. NIH 85-23, revised 1996, Office of Science and Health Reports, Bethesda, Maryland). The study was approved by the institutional ethics committee for animal research.

Procedure

Procedures were performed under general anesthesia with intravenous tiletamine–zolazepam–ketamine–xylazine combina-

☆ Preliminary results from this work were presented in a poster form at 21st European Congress of Pathology and published in an abstract form in Virchows Arch, 2007, 451 PP3-315. Clinically relevant results of the study were published as a full length article in Cardiology, 2009, 112:98–106.

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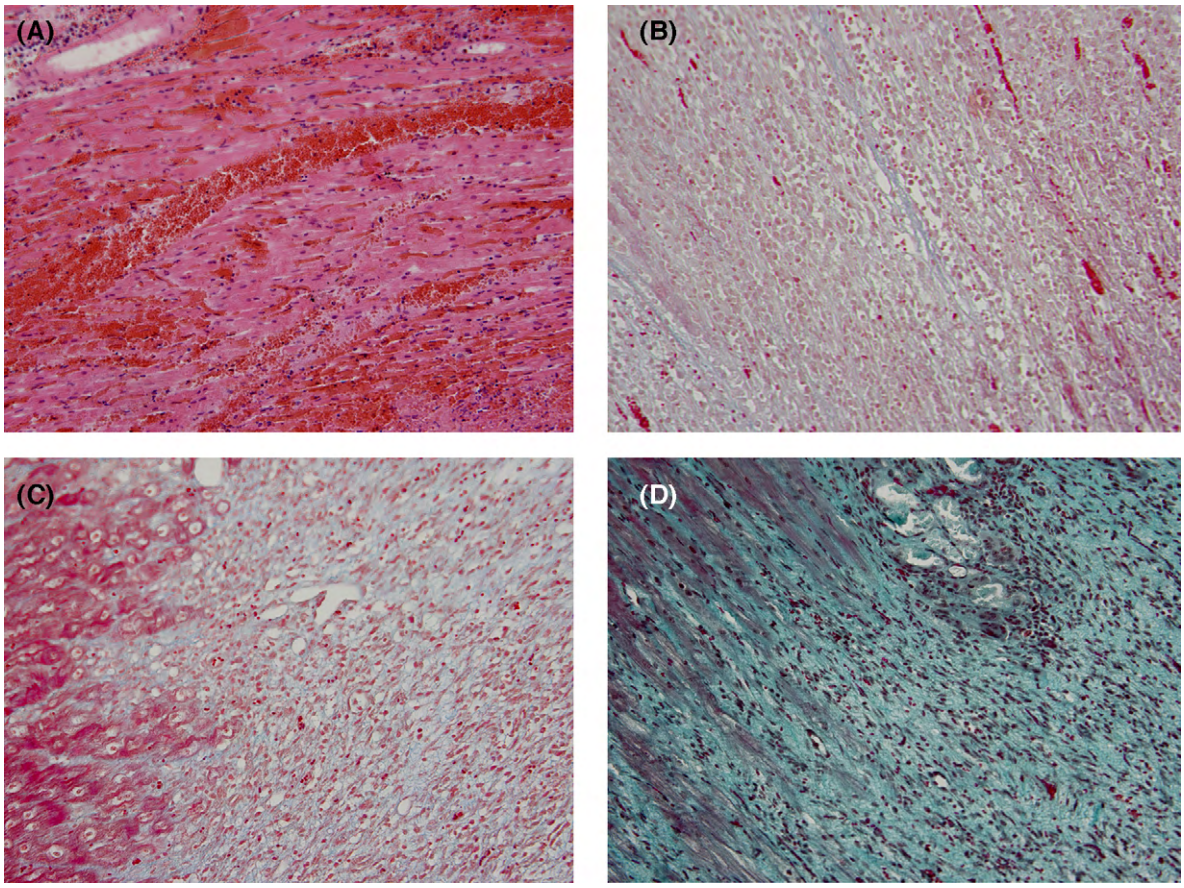

Fig. 1. (A) 24 h, coagulative necrosis, leucostasis, passive hyperemia, H+E 100×. (B) 3 days, scanty collagen fibers deposition, Mallory 100×. (C) 5 days, granulation tissue and collagen tissue, Mallory 100×. (D) 7 days, collagen tissue and large extracellular calcium deposits, Mallory 100×.

tion (2 mg/kg of each, mixed in the same syringe), followed by infusion of propofol (Propofol, Fresenius, 0.1–0.3 mg/kg min) and ventilation with a mixture of air and oxygen [3].

Experimental antero-apical AMI was created in all animals. The left anterior descending (LAD) coronary artery was catheterized under flouroscopic guidance with a 6F Amplatz right catheter passed through a femoral artery sheath. An angioplasty balloon was located in the middle part of the LAD artery. Anterior AMI was created by 90-min balloon inflation to 6 bar pressure. Animals were pretreated with oral metoprolol at a dose of 3 mg/kg given 20 h before AMI induction. Anticoagulant therapy was conducted using acetylsalicilate at a dose of 1 mg/kg pig body weight as a bolus given intravenously, along with heparin 180 U/kg put into the saline infusion just before the onset of balloon inflation. In addition, bolus of heparin at a dose of 180 U/kg of body weight was added intravenously at 45 min of balloon occlusion. Fluid balance was maintained with an infusion of 0.9% saline solution (5–10 ml/kg h) [5].

A continuous electrocardiogram was recorded during the experiment.

A large antero-apical myocardial dysfunction was verified by contrast ventriculography immediately after the 90-min balloon inflation [5].

The animals were euthanatized after 24 h, 3 days, 5 days, and 7 days. The hearts were eviscerated and dissected immediately after the procedure. Samples from infarcted tissue, macroscopically normal myocardium remote from the left ventricle and samples of the right ventricle, were immediately fixed in buffered formalin for 72 h and then routinely processed. After embedding in paraffin blocks, sections (5 μm) were cut and stained with hematoxylin–eosin, Mallory staining, and von Kossa staining.

Microscopic evaluation and photographic documentation were performed using Olympus BX45 microscope (Olympus Optical, Tokyo, Japan) equipped with 2×/0.08, 4×/0.13, 10×/0.30, 20×/0.50, 40×/0.75 and 60×/0.90 objective lenses, and with digital camera Olympus DP50. Olympus Viewfinder Lite™ software was used to acquire and process images.

Results

Macroscopically, red infarct, usual in ischemia with reperfusion, was observed.

Microscopically, after 24 h, coagulative necrosis with leucostasis was found. Passive hyperemia corresponded to red infarct (Fig. 1A).

After 3 days, granulation tissue with rare collagen fibers was recognized, proven by Mallory staining (Fig. 1B).

After 5 days, well-developed granulation tissue with collagen tissue deposition was present (Fig. 1C). After 7 days, the collagenous tissue was forming the scar (Fig. 1D).

A well-described phenomenon of contraction bands related to reperfusion was observed.

On the fifth day of AMI, dense deposits of intracellular and extracellular calcium were found, with maximum deposits seen on the 7th day. Von Kossa staining was used to prove calcium ions (Fig. 2A–D). Deposits were identified within the altered myocytes but not in the granulation tissue in the surroundings (Fig. 2A). In some areas, transition from intracellular deposits to extracellular deposits was obvious (Fig. 2B). Large extracellular deposits