Hypoxic reoxygenation during initial reperfusion attenuates cardiac dysfunction and limits ischemia–reperfusion injury after cardioplegic arrest in a porcine model

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Objective: In clinical practice, reperfusion of ischemic myocardium usually occurs under high arterial oxygen levels. However, this might aggravate cardiac ischemia–reperfusion injury caused by excessive oxidative stress. In an experimental in vivo study, the cardioprotective role of hypoxic reoxygenation during initial reperfusion was assessed.

Methods: Twenty-one adult pigs were started on cardiopulmonary bypass with aortic crossclamping (90 minutes) and cardioplegic arrest. During initial reperfusion, 10 pigs underwent standard hypoxic reoxygenation (Pao_2, 250–350 mm Hg), whereas gradual reoxygenation (Pao_2, 40–90 mm Hg) was performed in 11 pigs. Cardiac function was analyzed by means of the thermodilution method and conductance catheter technique.

Results: In both groups cardiac index was decreased 10 minutes after cardiopulmonary bypass compared with preoperative values. Sixty minutes after cardiopulmonary bypass, cardiac index improved significantly after gradual reoxygenation compared with that after hypoxic reoxygenation (3.2 ± 0.6 vs 2.5 ± 0.5 L $\cdot$ min^{-1} $\cdot$ m^{-2}, $P = .04$). Correspondingly, end-systolic pressure–volume relationship and peak left ventricular pressure increase were significantly less decreased in the gradual reoxygenation group. During and after reperfusion, malondialdehyde and troponin T values within the coronary sinus were significantly lower after gradual reoxygenation (60 minutes after declamping: malondialdehyde, 7.6 ± 0.8 vs 4.6 ± 0.5 μmol/L [$P = .007$]; troponin, 0.12 ± 0.02 vs 0.41 ± 0.12 ng/mL [$P = .02$]).

Conclusion: Hypoxic reoxygenation at the onset of reperfusion attenuates myocardial ischemia–reperfusion injury and helps to preserve cardiac performance after myocardial ischemia in a pig model.

Myocardial ischemia–reperfusion (IR) injury is a complex pathophysiologic process that is initiated at the very early moments of reperfusion and restoration of coronary blood flow after ischemia. The underlying mechanisms of myocardial IR injury have not been fully elucidated, and there are several factors playing an important role in the pathogenesis of IR injury.[1,2] Clearly, one of the major contributors to myocardial IR injury is the rapid generation of free oxygen radicals during early reperfusion of the ischemic heart.

Although the pathogenic effects of hyperoxia on cellular metabolism are well known,[3] the conventional method of conducting cardiopulmonary bypass (CPB) with cardiac arrest is to maintain high oxygen levels. The relationship between oxygen-induced radical production and impairment of myocardial energy metabolism, as well as myocardial dysfunction, after reperfusion was clearly shown.[4-6] In addition, it could be demonstrated that oxidative stress aggravates postischemic myocardial stunning.[7,8]

Clinically, the issue of IR injury concerns both cardiac surgeons and cardiologists because of the fact that myocardial reperfusion injury occurs in a setting of global ischemia in cardiac surgery, as well as more regionally in patients undergoing percutaneous coronary interventions for acute ischemia. Recently evolving postconditioning strategies describe multiple cardioprotective modalities to attenuate IR injury whereby hypoxic postconditioning reduces reoxygenation-induced injury.[9,10] However, there is still a lack of experimental in vivo studies investigating the effect of such cardioprotective strategies on cardiac function in complex heart surgery, as well as interventional cardiology, with the aim of routine clinical application.[11]

The present porcine in vivo study uses hypoxic reoxygenation (HR) to limit myocardial IR injury. We hypothesize that gradual reoxygenation (GR) on aortic declamping reduces IR injury after prolonged myocardial ischemia caused by decreased oxidative stress and myocardial injury.

MATERIALS AND METHODS

Study Design

We used an acute porcine model of CPB with 90 minutes of aortic clamping and cardioplegic arrest, 30 minutes of reperfusion, and 60 minutes of observation time.

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Abbreviations and Acronyms

CPB = cardiopulmonary bypass
EDPVR = end-diastolic pressure–volume relationship
ESPVR = end-systolic pressure–volume relationship
GR = gradual reoxygenation
HR = hypoxic reoxygenation
IR = ischemia–reperfusion
MDA = malondialdehyde

Twenty-one German landrace pigs were operated on with a mean body weight of 47.8 ± 3.5 kg. The animals were randomly divided into 2 groups. Ten pigs underwent HR (Pao2, 250–350 mm Hg) according to usual standards during the first 10 minutes of reperfusion. In 11 pigs GR was started for 2 minutes at hypoxic levels (Pao_2, 40–50 mm Hg) before declamping and continued for a consecutive 10 minutes at higher oxygen levels (Pao_2, 50–90 mm Hg) after aortic declamping. After the first 10 minutes of reperfusion, normoxic conditions were established again in both groups.

Experimental Setting

The animals were premedicated with intramuscular application of ketamine (30 mg/kg; Ketavest; Pharmacia, Erlangen, Germany), xylazine (2 mg/kg; Rompun; Bayer AG, Leverkusen, Germany), and midazolam (0.1 mg/kg; Dormicum; Roche Pharma AG, Reinach, Switzerland). General anesthesia and relaxation were achieved by administering fentanyl (1.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$; B. Braun AG, Melsungen, Germany), propofol (2 mg/kg for initiation and 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for maintenance; Disoprivan 2%; Astra Zeneca GmbH, Wedel, Germany), and pancuronium (0.1–0.2 $mg \cdot kg^{-1} \cdot h^{-1}$; Curamed Pharma GmbH, Karlsruhe, Germany).

After tracheotomy, the pigs were started on a volume-controlled respirator (EVITA 2; Fa. Dräger, Lübeck, Germany). The left internal jugular vein and left femoral artery were dissected for catheter placement. A thermodilution catheter (Baxter Healthcare Corp, Edwards Division, Santa Ana, Calif) was placed through the right internal jugular vein and advanced through the right atrium into the pulmonary artery. A cardiac output monitor (Model 9520A; Baxter Healthcare Corp, Edwards Division) was used for hemodynamic assessment. The conductance catheter was inserted through the right carotid artery and positioned through the aortic valve in the apex of the left ventricle. Data were evaluated with a Cardiac Function Analyzer (Leycom CFA-512; Leyden, The Netherlands). Monitoring included electrocardiography and arterial blood pressure, central venous pressure, pulmonary artery pressure, and blood temperature measurement (Marquette Solar 8000; GE Yorkshire, United Kingdom). Arterial blood gas, electrolyte, and hemoglobin levels were determined with a blood gas analyzer (Blood Gas System 288; Ciba–Corning, Medfield, Mass). Blood gases were maintained at physiologic pH, $Paco_2$ at 30 to 40 mm Hg, and Pao_2 at 90 to 120 mm Hg before and after CPB.

After induction of anesthesia and instrumentation, median sternotomy was performed in the usual manner. After opening of the pericardium, the left anterior descending coronary artery was dissected in its middle segment, and a 2.0-mm flow probe was placed (Transonic T206; Transonic Systems, Inc, Ithaca, NY) for coronary flow measurements with an ultrasound device (Transonic Flow-QC; Transonic Systems, Inc). A coronary sinus catheter was placed for taking blood samples.

The study protocol was approved by the Committee for Animal Research at the JW Goethe University, Frankfurt/Main, Germany. The study was performed according to the guidelines for animal experiments in Germany (Operative Eingriffe bei Versuchstieren formulated by the German Society for Animal Science and the ''Guide for the care and use of laboratory animals'' prepared by Gesellschaft für Versuchstierkunde, published 2001).

Cardiopulmonary Bypass

Systemic heparinization was administered (350 IU/kg body weight; Liquemin Roche, Grenzach–Wyhlen, Germany), and activated coagulation time during CPB was adjusted to greater than 400 seconds (Hemo Tec, Inc, Eaglewood, Colo). The aorta and right atrium were cannulated with an 18F flexible arterial cannula (Medtronic AG, Düsseldorf, Germany) and a 32F 2-stage venous cannula (DLP, Medtronic AG). A complete preconnected tubing set with membrane oxygenator (HILITE 7000) and cardiotomy reservoir (MV420; Medos Medizintechnik AG, Stolberg, Germany) was used with a standard roller pump (Fa. Stöckert). Priming volume consisted of Ringer solution (1600 mL) and 5000 IU of heparin. During CPB, arterial oxygen levels were continuously measured with an inline oxygen determination device (CDI 500; Cardiovascular Devices, Inc, Irvine, Calif).

All animals were started on normothermic (36°C–38°C) CPB for 120 minutes. After aortic crossclamping of 90 minutes, animals were weaned from CPB after 30 minutes of reperfusion. Cardiac arrest was initially achieved by means of a 2-minute antegrade infusion of warm blood cardioplegic solution, as described by Calafiore and colleagues,[12] and repeated every 20 minutes. Perfusion pressure during CPB was maintained at 50 to 60 mm Hg. After 30 minutes of reperfusion and weaning from CPB, an observation period of 60 minutes followed for hemodynamic measurements and taking blood samples, as mentioned below. At the end of the experiment, animals were killed.

End Points

Hemodynamic parameters. At steady-state conditions, the following parameters were measured before and 10, 30, and 60 minutes after CPB by using the thermodilution method: cardiac index, systemic vascular resistance index, pulmonary vascular resistance index, left ventricular stroke work index, and left ventricular end-diastolic pressure.

By using the conductance catheter technique, left ventricular performance was evaluated by determining the following parameters[13]: end-systolic pressure–volume relationship (ESPVR, end-diastolic pressure–volume relationship (EDPVR), and peak left ventricular pressure increase. The ESPVR and EDPVR were obtained from reduction of the preload by intermittent vena caval occlusion in a linear model. The conductance catheter system was calibrated by means of injection of hypertonic saline into the pulmonary artery.

Coronary blood flow. Coronary flow measurements of the left anterior descending coronary artery were performed before and 10, 30, and 60 minutes after CPB.

Number of defibrillations. During the reperfusion period of 30 minutes, the number of defibrillations was assessed. In case of ventricular fibrillation, the heart was defibrillated with 10 J (Lifepack 9; Physio-Control Corp, Redmond, Wash).

Myocardial ischemic damage. The troponin T concentration was assessed in micrograms per liter (Roche Diagnostics GmbH, Mannheim, Germany) in the coronary sinus blood before CPB and 20 and 60 minutes after aortic declamping.

Oxidative stress (lipid peroxidation). The concentration of malondialdehyde (MDA) in coronary sinus blood was assessed in blood samples obtained before CPB, as well as 10, 20, and 60 minutes after aortic declamping. Blood was stored on ice before centrifugation (4°C and 2500*g* for 10 minutes) for a maximum of 10 minutes in precooled ethylenediamine tetra-acetic acid tubes. Plasma samples were rapidly frozen at –80°C for later analysis. Two hundred microliters of plasma supernatant was used to determine MDA levels by using a spectrophotometric method with the Lipid Peroxidation Assay Kit (Calbiochem, San Diego, Calif).

Statistics

Statistical analysis was performed by using the BIAS software package (version 8.4.2) provided by Johann Wolfgang Goethe University, Frankfurt/Main, Germany. Differences between groups were tested with a 2-tailed