Video Article

Improvement of a Closed Chest Porcine Myocardial Infarction Model by Standardization of Tissue and Blood Sampling Procedures

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Abstract

Myocardial ischemia reperfusion (I/R) injury contributes to almost half of the necrotic area after myocardial infarction. To date there is no approved drug to prevent or reduce myocardial I/R injury. The study and understanding of the pathophysiological mechanisms of myocardial I/R injury is essential to develop successful treatments. Large animal experiments are an important step in translational methods. The porcine model of acute myocardial infarction has been established and described by ourselves and others. We aimed to further improve the value of the model by focusing in detail on the sampling techniques for use in future experiments. Furthermore, we emphasize small but important steps that can affect the quality of the final results. To mimic the clinical situation of myocardial I/R injury, a percutaneous coronary intervention (PCI) catheter was inserted into the left anterior descending coronary artery (LAD) of an anesthetized pig. °°°This model mimics acute myocardial infarction and PCI treatment in humans with the possibility of accurately determining the area at risk as well as the necrotic- and viable ischemic tissue. Here the model was used to investigate the effect of a bicyclic peptide inhibitor of FXIIa. The model can also be modified to allow longer reperfusion times to study later effects of myocardial infarction.

Video Link

The video component of this article can be found at https://www.jove.com/video/56856/

Introduction

Ischemic heart disease, in particular acute myocardial infarction (MI), is the main cause of death in developed countries [1]. Today, the standard treatment of MI is percutaneous coronary intervention (PCI), the balloon catheter treatment. One of the critical factors that affects quality of life and prognosis of patients after PCI-treated acute MI is the infarction size. The reduction of the size can have a great impact on patient survival and prognosis [2]. Myocardial ischemia/reperfusion (I/R) injury has a significant influence on the infarction size so one of the main aims in cardiovascular research is to prevent or reduce myocardial I/R injury [3]. The exact mechanisms of I/R injury are still under investigation [4]. Activation of the plasma cascades and endothelial cells are hallmarks of I/R injury [5]. Activation of the coagulation system is clearly involved [6,7]. Recently, the role of FXII, as an early upstream peptide involved in contact phase activation of the coagulation cascade, has been shown in a FXII knock out rat model of cerebral I/R injury [8]. Validation of these results in a porcine model is an important step into clinical translation. Therefore, we are testing a novel bicyclic (80 kDa protease) FXIIa inhibitor in the context of myocardial I/R injury in a pilot study.

Animal models, which mimic the clinical situation of acute MI and PCI treatments, are essential to improve our understanding of the pathophysiology of myocardial I/R injury and to test novel treatment options. Pigs represent a good animal model for clinical myocardial I/R injury. This is not only because their hearts are very similar to human hearts with respect to anatomy and coronary circulation, but they also show similar pathophysiological responses to myocardial ischemia and reperfusion [9,10]. Other models such as rats and mice do not fulfill these criteria and show considerable differences when compared to human hearts [11,12], whereas dogs for example, have many more collateral coronary vessels as compared with humans [13].

The porcine acute myocardial infarction model has been widely used in cardiovascular research to investigate ischemic heart disease including myocardial I/R injury [14,15,16,17]. The latter is an inflammatory condition, because of which minimizing the inflammatory reaction related to sternotomy or thoracotomy used in open-chest surgery is essential. The closed chest model using a clinical C-arm angiography setting overcomes this problem. Furthermore, one of the most important points is that our protocol provides an accurate distinction between ischemic (area at risk, AAR) and non-ischemic areas of the left ventricle (area not at risk, ANR) so that the infarct size (necrotic ischemic tissue, NIT) can be accurately determined. Our aim for this paper is to clearly define a reproducible methodology of a porcine myocardial I/R injury model, in particular with respect to myocardial tissue sampling, which will allow for a more precise analysis of the molecular mechanisms of I/R injury and a clearer picture of the effects of novel drug treatments.

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Protocol

1. Animals:

All animals were treated according to the guidelines of the Swiss national laws. The study was approved by the local animal experimentation committee of the Canton of Bern (permission no. BE 25/16).

Use large white pigs of both sexes (~30 ± 5 kg). Divide the animals blindly into two groups, one group receiving a bicyclic (80 kDa protease) inhibitor of FXIIa or treatment of choice and the other an inactive control.

2. Surgical procedure (Figure 1)

1. **Anesthesia and preparation of the animal:**
    1. Fast the animals for 12 h before starting the experiment.
    2. Pre-medicate the animal with 20 mg/kg Ketamine and 2 mg/kg Xylazine via an intramuscular injection into the neck, using a 10 mL syringe. Record the animal weight and sex.
    3. Induce anesthesia by injecting 0.5 mg/kg Midazolam and 0.05 mg/kg Atropin into the auricular vein. Intubate the animal with an endotracheal tube.
    4. Maintain the anesthesia by mechanical ventilation using a respirator (O_2/air 1:3, Sevorane 1.5%), a 7 - 8 mm airway tube and a filter. Adjust the fraction of inspired oxygen (FiO_2) to 35% and the tidal volume to 6-10 mL/kg.
    5. Depth of anesthesia is assessed as adequate in absence of either motor or autonomic responses to nose pinching. Palpebral reflexes and jaw tone were monitored continuously as well targeting relaxed jaw tone and absence of palpebral reflexes. Core temperature was continuously monitored and maintenance of normothermia (38-38.5 °C) was ensured with passive warming (isolating blankets and warm bottles).
    6. Dissect free, as previously described by Koudstaal and his colleagues steps 3-1 to 3-3[18], the carotid arteries on both sides and cannulate them with a 7F sheath. Cannulate the left jugular vein with a 7F sheath for venous blood sampling.
    7. Administer a bolus dose of 250 µg Fentanyl analgesic through the central venous line followed by 250 µg/h as a continuous intravenous infusion using an infusion pump. Monitor body temperature, heart rate and rhythm with a 3-lead electrocardiogram (ECG), arterial and central venous pressure during the whole experiment.
    8. Using standard blood collection tubes, withdraw the following baseline blood samples from the venous line: 5 mL citrated plasma and 2.9 mL EDTA plasma into the respective tubes. Centrifuge immediately at 2,000 x g for 15 min at 4 °C. Take 2.9 mL blood into a serum tube and allow to coagulate for 30 min at room temperature before centrifuging as described above.
    9. Aliquot 200 µL of plasma or serum into 500 µL tubes and store all samples at -80 °C for further analysis.
    10. Withdraw 0.5 mL of blood from the arterial line using the special blood gas analysis (BGA) syringes to measure BGA using the BGA machine according to the manufacturer's instructions.
    11. Withdraw 0.5 mL of blood from the venous line using a standard 2 mL syringe and immediately transfer it into the ACT cartridge using a 30 G needle. Insert the filled cartridge into the ACT machine to measure clotting time according to the manufacturer's instructions. See **Figure 2** for time points.
    12. Administer 5000 IU unfractionated heparin into the venous line using a 2 mL syringe and allow the animal to stabilize for 20 min before starting the MI experiment.
    13. Monitor ACT every 30-45 min as mentioned in 2.1.11. Inject 2500 IU unfractionated heparin intravenously if ACT is < 180 s.

2. **Myocardial infarction experiment**
    1. Use a standard C arm fluoroscopy equipment (coronary angiography program, angle 0°, 12 frames per second, ~70 kV) - or alternatively a dedicated angiography system - to perform the coronary intervention.
    2. Use fluoroscopic guidance to insert a pressure catheter (5F, 120 cm) via the previously placed sheath in the left carotid artery. Advance it into the left ventricle. Connect the pressure catheter to an acquisition system to record left ventricular pressure. The acquisition system needs to continuously calculate and record heart rate, developed pressure, dP/dt maximum (contractility of left ventricle) and dP/dt minimum (relaxation of left ventricle) during the entire experiment. Record baseline values for 10 min.
    3. Insert a 6F (100 cm, EB3.75) guiding catheter via the previously placed sheath into the right carotid artery. Advance it to the left coronary artery to reach the left anterior descending coronary artery (LAD) under X-ray guidance. Inject contrast medium using a 20 mL syringe via the guiding catheter to perform a baseline coronary angiography.
    NOTE: Injection of contrast medium was done by manual pressure but a dedicated power injector system could be used as well.
    4. Assess the size of the LAD on the X-ray monitor to select the appropriate size of the percutaneous coronary intervention (PCI) catheter. Use PCI balloons with a length of 15-25 mm and diameters between 2 and 3.5 mm depending on the LAD size. Assemble PCI catheter and coronary guidewire (F 014/J, 175 cm). Connect the PCI catheter with the inflation device pre-filled with contrast medium.
    NOTE: The LAD diameter can be measured directly on a calibrated monitor using a ruler and the size of the PCI balloon is then selected accordingly.
    5. Insert the assembled system from 2.2.4 into the lumen of the guiding catheter. Advance the guidewire into the LAD until it reaches beyond the second diagonal branch of the LAD.
    6. Use fluoroscopic guidance to advance the PCI catheter until it reaches about the middle of the LAD. Choose the LAD blocking site depending on the anatomy of the coronaries, usually after the second, sometimes after the first diagonal branch (**Figure 3**) in order to have similar percentages of the AAR of the left ventricle (LV).
    NOTE: The choice of the blocking site depends on the length of the diagonal branches and thus the area of tissue which is supplied by blood via the respective branch. In case of a long, bifurcated first diagonal branch the blocking site will be just after this. In case of a shorter first diagonal branch, the blocking is done after the second diagonal.

7. Remove the guidewire and then increase the pressure in the inflation device to 7-10 bar to inflate the balloon of the PCI catheter and induce myocardial ischemia for 1 h. Gradually increase the FiO_2 to 50-60% between 15 and 40 min of ischemia. Keep tidal volume at 6-10 mL/kg.
   NOTE: This procedure will reduce the occurrence of extrasystoles and decrease the frequency of ventricular fibrillations.
8. Record a 5-10 s video sequence while injecting contrast medium through the guiding catheter to have an angiogram of the balloon of the PCI catheter in place just after starting ischemia; repeat after 10 min of ischemia to verify complete occlusion of the LAD distal to the balloon of the PCI catheter.
9. Monitor the animal closely to immediately detect and treat (2.2.10) cardiac arrhythmias. Extrasystoles usually occur and increase in frequency (>3 per min) between 20 and 40 min after induction of myocardial ischemia. If this occurs, gently massage the neck on both sides just below the cheek. In most cases this will be sufficient to re-establish a regular heartbeat, probably by stimulation of the vagal nerve baroreceptors located on the common carotid artery.
10. If cardiac arrhythmias progress into ventricular fibrillation, use an external, biphasic defibrillator to re-establish a sinus rhythm. Apply 5-10 chest compressions using the defibrillator pads immediately before applying the shock in order to fill the coronaries with oxygenated blood and then shock with 150 J (for 30 kg animals).
11. Repeat if necessary and increase the energy to 175 J after the 3rd shock. Use higher energy settings for heavier animals.
12. Five min before the end of the ischemia time repeat the blood sampling mentioned in 2.1.8-2.1.13. Inject the test substance (either the bicyclic FXIIa inhibitor or control 4 mg/kg, do this this blindly) intravenously through the central venous line and flush the line with 20 mL saline.
13. Withdraw one plasma citrate sample (as mentioned in 2.1.8 and 2.1.9) 4 min after injecting the substance in 2.2.12.
14. Perform an angiogram (2.2.3) to confirm LAD occlusion, then deflate the balloon of the PCI catheter and remove this catheter from the guiding catheter. Confirm perfusion of the LAD distal to the occlusion site by angiogram immediately after deflation and removal of the balloon of the PCI catheter, 10 min thereafter, whenever signs of myocardial ischemia were visible by ECG for more than 5 min, and immediately before re-occlusion of the LAD.
15. Allow reperfusion of the ischemic myocardium for 2 h. Take plasma and serum samples at 10, 30, 60 and 115 min of reperfusion (2.1.8 and 2.1.9). Monitor BGA at 60 and 115 min (2.1.10).
16. Reinsert the PCI catheter together with the guidewire to exactly the same position as used for the ischemia. Inflate the balloon of the PCI catheter as before and confirm LAD occlusion by angiogram (2.2.3). Remove the pressure catheter from the left ventricle and stop recording.
17. Inject 100 mL 2% Evans Blue in phosphate buffered saline (PBS, pH 7.4) into the central venous line. About 30 s later, when the whole animal turns blue, inject 40 mL 20% KCl to euthanize the animal. Death was confirmed by the absence of ECG signals and pulse waves.

3. Sampling techniques

1. **Extracting, dissecting and sampling the heart (figure 4)**
   1. Perform a sternotomy to expose the heart. Follow the protocol previously described by Koudstaal and colleagues, steps 8-2 and 8-3[18]. Cut open the pericardium while inspecting for abnormalities, which might stem from earlier pericarditis, and exclude the animal from further evaluation.
   2. Deflate and remove the PCI as well as the guiding catheter. Excise the heart for further analysis. Cut the vena cava and remove blood using a suction pump, then cut all the large vessels connecting the heart with the body.
   3. Rinse the heart inside and out with room temperature saline. Weigh the whole heart.
   4. Within 30-40 min, cut the heart into slices of about 3-5 mm from the apex to the *Chordae tendinae* of the mitral valve, perpendicular to the long axis using a sharp knife.
   5. Be careful to always place the heart in the same orientation with the ventral side facing up in order to keep the orientation of the cut samples (**Figure 5**).
   6. Photograph the slices of the heart using a digital single lens reflex camera.
   7. Cut away the right ventricle (discard as not needed). Photograph the left ventricle slices and weigh all the slices for the total weight of the left ventricle.
   8. Differentiate between the Evans Blue positive and Evans Blue negative tissue in all the sections. Dissect the slices to separate the ischemic (Evans Blue negative) from the non-ischemic tissue (Evans Blue positive) using a scalpel.
   9. First analyze the Evans Blue negative sections (the ischemic area at risk, AAR). Weigh them all and put them all into a plastic container.
   10. Cover the slices entirely with 100-150 mL (according to the heart size) triphenyl tetrazolium chloride solution (2 g TTC, 16 g Dextran, molecular weight 48000-90000, in 200 mL PBS, freshly prepared) so that the heart pieces can move freely inside the solution. Cover the container and incubate for 20 min at 37 °C while gently shaking.
   11. During this 20 min incubation time weigh the Evans Blue positive pieces (area not at risk, ANR), take samples for Tissue-Tek embedding (choose the most distal part from the injury) and store at -80 °C for further analysis. Transfer the rest into 4% formaldehyde solution and store at room temperature for histology sections.
   12. Remove the pieces of the AAR from the TTC solution. The red stained tissue is viable ischemic tissue (VIT) and the non-stained tissue is necrotic ischemic tissue (NIT). Cut 2 small pieces (blocks of 2-3 mm) from both the NIT and VIT, embed them into Tissue -Tek and store at -80 °C for further analysis. These samples should have the same weight.
   13. Fix the rest of the pieces (all slices derived from the AAR) by pinning them down in a Styrofoam container and covering completely with 4% formaldehyde solution for 24 h at room temperature in a fume hood. The pieces should stay flat for the photographic documentation in the next step.
   14. The next day photograph both sides of the pieces with a high-resolution camera with the same zoom setting and distance from the tissue (same magnification). Add automatic scale bars to all pictures. All bars need to have the same length.
       NOTE: The high-resolution camera should be connected to software that automatically adds the scale bar to each photo.

2. **Calculation of the AAR and the infarct size**
    1. %AAR of left ventricle = (weight of AAR in g/ weight of left ventricle in g)* 100.
    2. Use ImageJ software to calculate the total surface area of both the AAR and NIT (both sides of each piece) based on the photographs.
    3. Adjust the scale bar by selecting the scale bar length using the straight line from the angle tool. Choose from the menu Analyze > Set Scale and insert the known distance and unit of the scale bar. Choose "global" so the same scale will be applied to all pictures.
    4. Mark the whole surface area of the tissue using the free hand selection tool to calculate AAR. Be careful not to include the side (height) of the tissue and/or the fatty tissue (figure 6-C).
    5. Set the measurement by choosing "area" and "display label" from Analyze > Set Measurements menu. Measure the surface area from Analyze > Measure.
    6. Repeat step 3.2.5 to measure NIT (mark only the non-stained tissue). Note: Don't include the fatty tissue (figure 6-D) in the NIT calculation. Repeat the step on the other side of the tissue.
    7. Calculate the average AAR and NIT for each piece of tissue.
    8. Use the values obtained from 3.2.7 to calculate the NIT as a percentage of the AAR:%NIT of AAR = (Σ average surface area of NIT in cm^2/ Σ average surface area of AAR in cm^2)* 100.
    9. Two different investigators should repeat the above method. The acceptable margin of difference is < 10%.

3. **Ischemia markers**
    1. Use the EDTA plasma samples, which have been previously stored at -80 °C (2.1.9) to measure the level of cardiac troponin-I using a single-plex Luminex-type assay as previously described [19].

Representative Results

One animal died prematurely before administration of FXIIa inhibitor or control peptide due to a technical error (sudden drop of blood pressure during ischemia time, before addition of test substance). One animal was excluded from the FXIIa inhibitor group because no ischemia/ reperfusion injury was observed due to abnormal anatomy of the left anterior descending artery (LAD). A large part of the left ventricle, including the apex, was perfused by the circumflex artery in this animal. The animals included in the final analysis were n = 2 in the bicyclic FXIIa peptide inhibitor group (mean weight of 27.5 ± 2.5 kg) and n = 3 receiving an inactive bicyclic control peptide (mean weight of 29 ± 0.8 kg).

X-ray video imaging / coronary angiography of the pig heart is used to visualize the position of the pressure catheter and to decide where to block the LAD (**Figure 3A**). **Figure 3B** shows the catheter position, blocking the blood flow distal to the second diagonal branch. Comparison of **Figure 3A** and **3B** also allows an estimation of which part of the LAD-supplied myocardium will be ischemic. At the end of the 2 h reperfusion period the PCI catheter is reintroduced and inflated at the same position as it was during ischemia. Evans Blue is then injected intravenously to accurately determine the AAR (**Figure 5A**). After excision of the heart, the left ventricle is sliced into 3-5 mm thick sections from the apex up to the mitral valve, perpendicular to the long axis. AAR and ANR are clearly demarcated by Evans Blue staining on the slices. AAR and ANR sampling areas are shown in **Figure 5B**.

The AAR, expressed as percentage of the LV, shows no statistically significant differences between the FXIIa treated group and the control group (**Figure 6A**). The infarct size (NIT/AAR) shows no differences between the groups either (using non-parametric Mann-Whitney test, $p > 0.05$, **Figure 6B**). These data suggest that FXIIa inhibitor alone, at the used concentration and duration of application, could not protect the heart from myocardial I/R injury**. Figure 6C and 6D** show how to mark the AAR and NIT borders in order to accurately and reproducibly measure the respective surface areas.

The blood sampling strategy allows the release of the cardiac muscle damage marker cardiac troponin-I to be monitored over time. There is almost no difference after one hour of ischemia with the baseline while after reperfusion there is a continuous increase over time as shown in **Figure 7**. For troponin-I, inter-group differences were also not significant in these experiments.

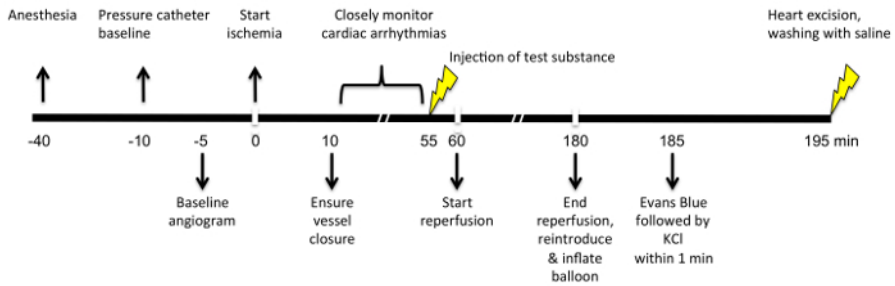

Figure 1. Overview of the experimental timeline. Schematic timeline for the important steps in the myocardial ischemia/reperfusion injury model. Baseline coronary visualization, starting time of the ischemia, monitoring cardiac arrhythmias and injecting the test substance are important steps in the experiment. The use of exact timing in all experiments ensures reproducibility. Euthanizing the animal and excision of the heart should be done within 15-20 min after completion of the 2h reperfusion phase. KCl: potassium chloride. Please click here to view a larger version of this figure.

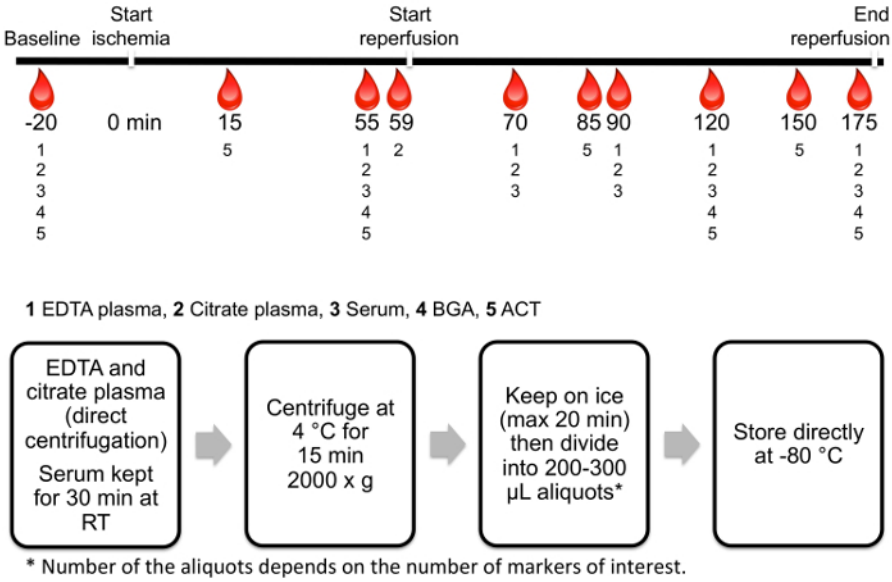

Figure 2. Timeline of blood sampling and analysis. Time points for blood sampling are indicated together with type of anticoagulant used. Additional samples can be taken according to the experiment and analytes to be measured. ACT: activated clotting time, BGA: blood gas analysis, RT: room temperature. Please click here to view a larger version of this figure.

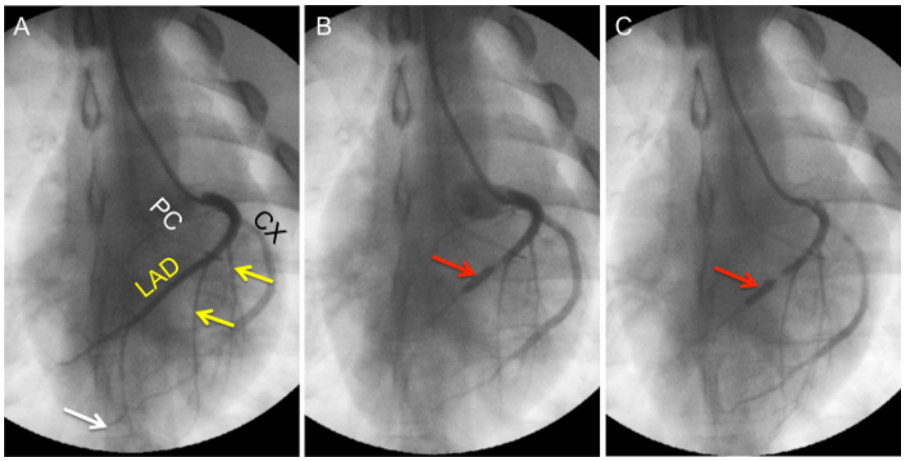

Figure 3. Coronary angiography. Fluoroscopic view of (A) the left coronaries at baseline, the yellow arrows point to the first and the second diagonal branches, the white arrow points to the heart apex (B) the occluded LAD showing the no flow area of the left ventricle (LV), the red arrow points to the PCI catheter (C) re-closure of the LAD at the end of the reperfusion with the balloon of the PCI catheter re-inserted to the same site in the LAD as during ischemia. CX: circumflex coronary artery, LAD: left anterior descending coronary artery, MC: Millar catheter, inserted in the left ventricle. Please click here to view a larger version of this figure.

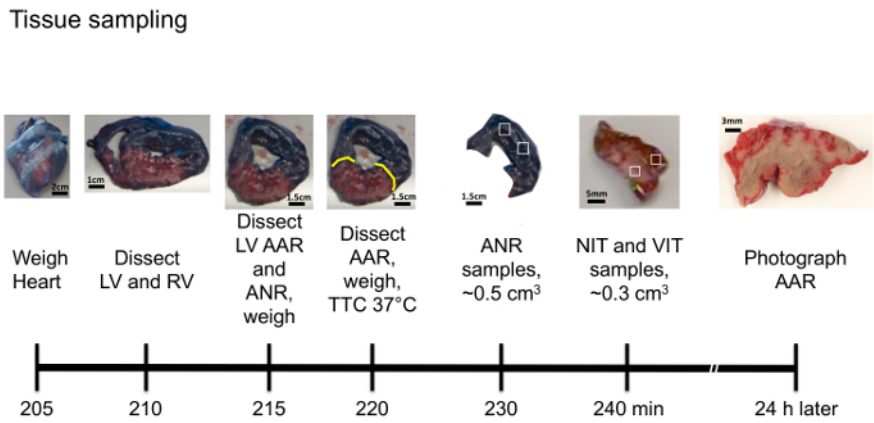

Figure 4. Schematic chart of tissue sampling. Exact timing of heart dissection and sampling of the different areas for further analysis. The timeline starts at 205 min after beginning of ischemia, 20 min after termination of the animal experiment. It is important to incubate the tissue sections in TTC within a maximum of 40 min after euthanizing the animal. Sampling of ANR, VIT and NIT is indicated as white squares. Incubating the AAR in 4% formaldehyde allows clear distinction between NIT and VIT for accurate determination of the infarct size. AAR: area at risk, ANR: area not at risk, LV: left ventricle, NIT: necrotic ischemic tissue, OTC: Tissue-Tek, RV: right ventricle, TTC: triphenyl tetrazolium chloride, VIT: viable ischemic tissue. Please click here to view a larger version of this figure.

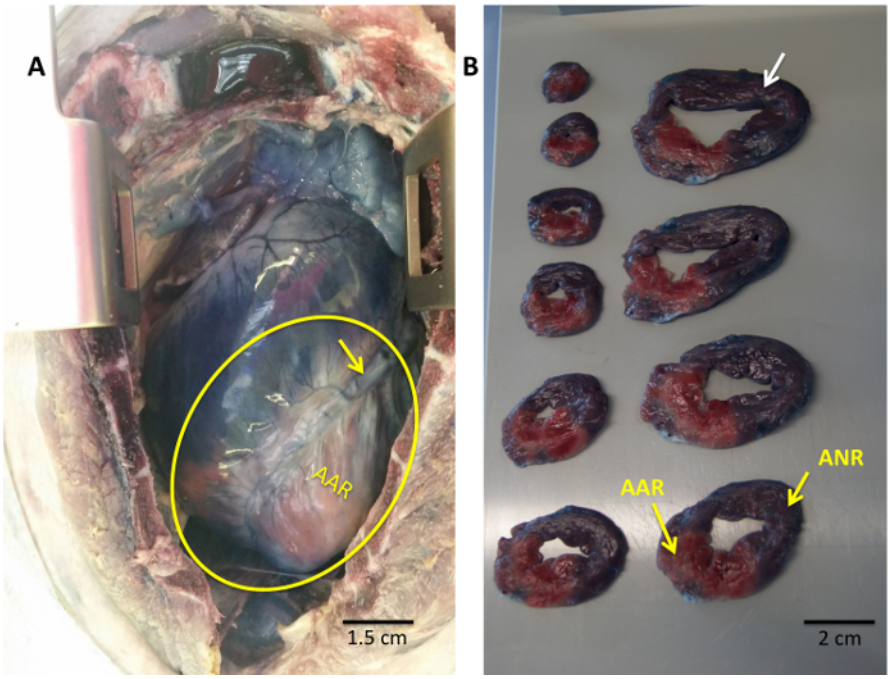

Figure 5. In-situ differentiation between area at risk (AAR) and area not at risk ANR. (A) Representative picture of the whole heart just after sternotomy at the end of the experiment. (B) Representative picture showing the 3-5 mm thick left ventricle slices after dissection. AAR and ANR are clearly defined, indicated by yellow arrows, and the white arrow shows the ANR sampling area. Please click here to view a larger version of this figure.

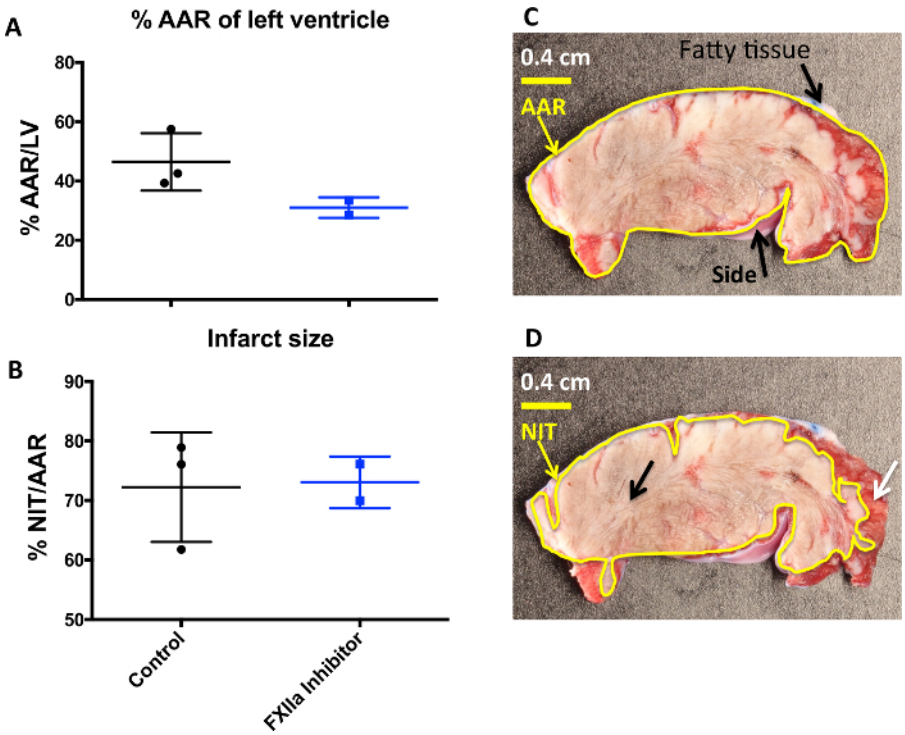

Figure 6. Ischemia and infarct size. (A) The percentage weight of the AAR of the left ventricle (LV). (B) The percentage surface area of the NIT of the AAR. (C) A representative picture of the AAR calculation. (D) A representative picture of the NIT calculation. The white arrow shows the VIT sampling area and the black arrow shows the NIT sampling area. Data were calculated using ImageJ software. Values are shown as dots for each individual experiment with indication of mean ±± SD. Control group, n = 3 and FXIIa inhibitor treated group, n = 2. Please click here to view a larger version of this figure.

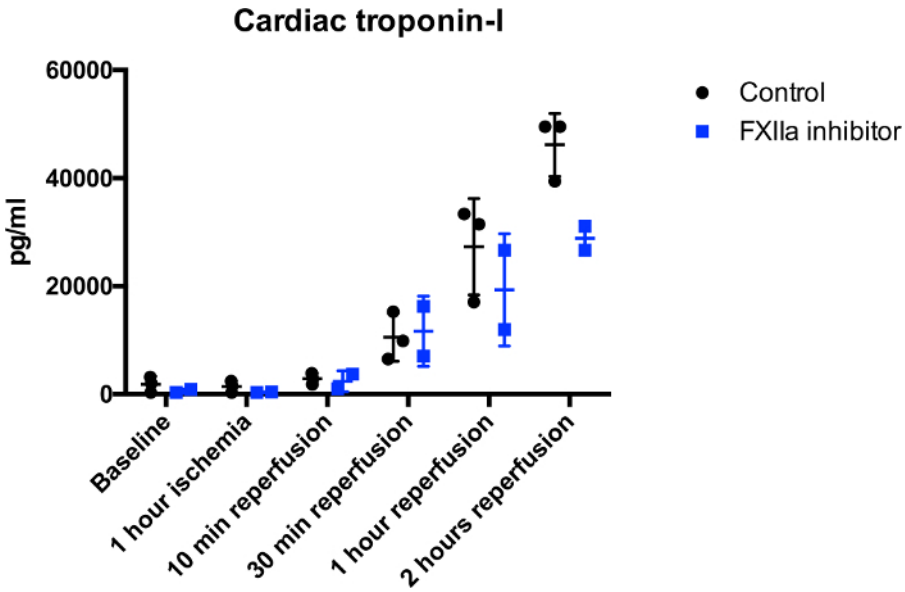

Figure 7. Cardiac muscle damage marker. Cardiac troponin-I concentration over time in pg/ml of both the control and FXIIa inhibitor treated group. Blood was collected from the jugular vein into EDTA plasma tubes at baseline, end of ischemia and several time points during reperfusion and cardiac troponin-I was measured by single-plex suspension array (Bio-Plex). Data are shown as dots for each individual experiment with indication of mean ±± SD. Control group, n = 3 and FXIIa treated group, n = 2. Please click here to view a larger version of this figure.

Discussion

Myocardial I/R injury has a significant effect on the final infarct size which is directly translated into the patient's prognosis after acute myocardial infarction[3]. Understanding the pathophysiology of myocardial I/R injury is the first step to reduce or prevent it. Myocardial I/R injury is an acute condition that occurs directly after reperfusion of the occluded vessels. I/R injury leads to activation of the innate immune response and cellular damage occurs at the site of reperfusion and the surrounding tissues[20]. A recent study showed an improvement in the neurological outcome in a rat model of brain I/R injury when treated with FXIIa inhibitor[8]. However, in the current pilot study we found no effect of the bicyclic FXIIa inhibitor

on myocardial I/R injury. The used FXIIa inhibitor is novel and its pharmacokinetics in pigs are not yet known. Therefore, the observed lack of effect might be caused by inappropriate dosing or application. This needs to be addressed in follow-up studies.

Standardizing an animal model is essential to investigate in depth the pathophysiology of myocardial I/R injury and to bring suitable solutions into clinics. Investigating the pathophysiology of myocardial I/R injury requires good and representative sampling in order to study the cellular mechanisms underlying it. The porcine closed chest myocardial I/R injury model provides a reproducible method, which is close to the clinical situation, and useful to help understanding the cellular mechanisms and test novel new therapeutics. Variants of the present model have been described before for the above mentioned purposes[14,17,18].

Our protocol of acute myocardial infarction in pigs does not need pre-treatment with amiodarone as previously described[18,21]. We used carotid sinus massage to reduce cardiac arrhythmias and a biphasic defibrillator for cardioconversion in case of ventricular fibrillation. The use of carotid sinus massage is clinically known to influence atrial fibrillation[22], but so far it has not been shown to prevent or delay the onset of ventricular fibrillation in MI, either in humans or in pig models. Moreover, the use of sevoflurane helps to reduce ventricular arrhythmias as well as mortality rate in the porcine model of acute myocardial infarction[23].

To ensure reproducibility and reduce the risk of thrombosis during the experiment, multiple doses of heparin were injected based on the repeated measurement of ACT, rather than using fixed heparin doses as described for example by Koudstaal et al[18]. A controlled amount of heparin administration helps to investigate the coagulation cascade in the context of I/R injury. Evans Blue allows accurate determination of AAR/LV. The intravenous injection of the Evans Blue after re-occlusion of the LAD at the exact site during ischemia induction under fluoroscopic guidance leads to blue staining of the whole pig including the non-ischemic part of the heart with minimum effect on the ANR myocardium and vasculature. Evans Blue is a known cytotoxic substance[24]. In the current experiments it was crucial to maintain the viability of the endothelial cell layer in ANR in the heart vasculature in order to use it as an intra individual control so 100 mL Evans Blue was injected systemically and diluted with the whole blood reducing its toxicity. Previously, in a similar setting, 50 ml 2% Evans Blue was injected directly into the coronaries increasing the risk of its cytotoxicity to cardiac cells[25]. The next important step was to dissect the heart directly into 3-5 mm slices from the apex up to the mitral valve (the exact position in every animal) and using this method to make an accurate calculation of the AAR as a percentage of the left ventricle.

The current description of the method provides finer details that have not previously been described. Incubating TTC stained section in 4% formaldehyde for 24 hours provides a clear distinction between viable (red) and necrotic (white) tissue, which finally increases the reproducibility of the sampling for further molecular staining. The blood sampling strategy over 2 h of reperfusion enables the detection of newly expressed molecules in the very early (10 and 30 min) stages of reperfusion as well as later (60 and 120 min). The correct blood and tissue sampling and storage are also crucial for the analysis of plasma cascade markers such as the expression of complement and coagulation proteins.

The current protocol can be modified to have a longer reperfusion time, from a few hours to days. This allows the researcher to investigate the later consequences of I/R injury on the heart and also enables the testing of novel drugs and assessment of their effects. The limitation of the current protocol is the use of a pressure-tip catheter for the measurement of heart function. More reliable data on cardiac function can be obtained by the use of a pressure-volume loop measurement system. In summary the current method provides detailed important steps required to increase the reproducibility of the porcine closed chest myocardial I/R injury model when the intended use of the model is to study the cellular and molecular changes in the context of studying myocardial I/R injury pathophysiology or studying novel therapeutic options.

Disclosures

The authors declare no conflict of interest.

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