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Imaging the myocardium at risk with ^{99m}Tc-lactadherin administered after reperfusion in a porcine model☆

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ABSTRACT

Introduction: Phosphatidylserine is translocated from the inner to the outer leaflet of the plasma membrane in the early stages of apoptosis and necrosis and in reversibly injured cells. In rabbit hearts, ischemia followed by reperfusion results in exposure of phosphatidylserine on myocytes unaffected by apoptosis or necrosis. Lactadherin was recently introduced as a highly sensitive phosphatidylserine ligand. We hypothesized that ischemic myocardial cell damage can be identified by radio-labeled lactadherin and that the ischemic area at risk (AAR) can be visualized retrospectively after reperfusion.

Methods: Left anterior descending coronary artery in pigs was occluded for 20 minutes, 45 minutes or 45 minutes preceded by ischemic preconditioning. In all three groups, ^{99m}Tc-lactadherin was injected intravenously 30 minutes after reperfusion. The AAR was demarcated by Evans blue and the infarct size by 2,3,5,-triphenyltetrazodium chloride staining.

Results: The regional myocardial uptake of ^{99m}Tc-lactadherin closely correlated with the AAR ($r = .83$, $P = .001$). The area of ^{99m}Tc–lactadherin uptake was unaltered by a shorter duration of ischemia and ischemic preconditioning ($P = .23$) despite significantly different infarct development ($P = .001$).

Conclusion: The results suggest that ^{99m}Tc–lactadherin can be used as a sensitive marker for AAR imaging when injected 30 minutes after reperfusion following acute ischemia.

1. Introduction

Apoptosis and necrosis contribute to myocardial cell death following coronary artery occlusion [1,2]. Through phagocytosis, apoptotic cells are removed by macrophages which recognize the cells due to the exposure of a phospholipid, phosphatidylserine (PS), on the cell surface [3,4]. PS is translocated from the inner to the outer leaflet of the plasma membrane in early stages of apoptosis and necrosis and in reversibly injured cells [4–6]. PS tracers can be used as markers of cell death and injury but cannot be considered suitable for distinguish between apoptotic, necrotic and reversible injured cells [7]. Annexin V is a cellular protein with binding affinity to membrane exposed PS [8]. Reversibly and irreversibly injured cells can be visualized by labeling annexin V with a fluorescent or radioactive moiety [9,10]. This technique has previously been successfully used to detect cell death in ischemic heart disease [11,12]. Lactadherin was recently introduced as a more sensitive protein ligand for detection of PS externalization because of its superior affinity to PS [13,14]. Lactadherin has been labeled with the Single Photon Emission Computed Tomography (SPECT) isotope ^{99m}Technetium, and *in vitro* binding to apoptotic cells has shown this agent's potential as a marker for apoptosis [15]. However, clinical or experimental studies with lactadherin or radiolabeled lactadherin in ischemic heart disease have not been performed.

Ischemic heart disease remains a leading cause of death, even in the era of immediate revascularization treatment for acute coronary syndromes [16]. Adjunctive cardio-protective therapies, e.g. local and remote ischemic preconditioning (IPC), are recognized as efficient mechanisms that protect against cardiac ischemic reperfusion injury [17,18]. Clinical trials using mortality as the endpoint require high numbers of patients and huge resources to demonstrate a beneficial effect of such new treatment [19]. To reduce the need for large population sizes, numerous trials have used surrogate endpoints, including imaging measures of the area at

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risk (AAR) and the infarct size (IS), to calculate myocardial salvage after coronary revascularization [20]. There is consequently a growing need for myocardial diagnostic imaging to accurately determine the size of the jeopardized myocardium at risk of infarction, i.e. the AAR.

The aim of our investigation was to evaluate ^{99m}Tc-labeled lactadherin as a new sensitive imaging tool for quantification of the AAR in a pig model with myocardial ischemia and reperfusion.

2. Materials and methods

2.1. Preparation of ^{99m}Tc-lactadherin

HYNIC-lactadherin was produced as described previously [15], whereas the labeling of HYNIC-lactadherin with ^{99m}Technetium was scaled up compared to other studies [21]. Briefly, 100 μL HYNIC-lactadherin (0.31–0.35 mg/mL protein, 1.0–1.8 HYNIC per protein molecule in citrate buffer, 20 mM, 100 mM NaCl, pH 5.2) was coupled with 150 MBq $^{99m}TcO_4^-$ in the presence of 10 μL tricine (100 mg/mL in citrate buffer, 20 mM, 100 mM NaCl, pH 5.2) and 5 μL of $SnCl_2$ (5 mg/mL in 0.05 N HCl). Radiochemical purity was higher than 95% and was measured by two thin layer chromatography methods using ITLC-SG as stationary phase and acetone or acid-citrate-dextrose buffer (0.068 M citrate, 0.074 M dextrose, pH 5.0), respectively, as the mobile phases. In the method using acetone the percentage of ^{99m}Tc-pertechnetate is estimated and in the method using acid-citrate-dextrose the overall radiochemical purity is determined. In addition to ^{99m}Tc-pertechnetate the latter method determines the contents of colloidal ^{99m}Tc and ^{99m}Tc-tricine.

2.2. Animal preparation and experimental protocol

The experiments were approved by the Danish Inspectorate of Animal Experiments and performed in accordance with their guidelines. Cardiac interventions were performed on 32 female Danish Landrace/Yorkshire pigs, weight 75 kg. Anesthesia was induced using a bolus of 15 mg/kg pentobarbital intravenously (Mebumal®, DAK, Copenhagen, Denmark) followed by a continuous infusion of 90 mg·h^{-1}. The pigs were intubated and ventilated (Datex Ohmeda s/5 Avance ventilator, GE Health Care, UK). An arterial sheath was placed in the right carotid artery for heart catheterization and arterial blood sampling. A venous sheath was placed in the corresponding vein for infusions. The pigs were heparinized (10,000 IU intravenously as bolus, followed by 2000 IU·h^{-1}) (Heparin®, Leo, Copenhagen, Denmark). Blood pressure was measured with a fluid-filled pressure transducer catheter connected to the arterial sheath. After initial preparation, the animals were stabilized for 20 minutes. Ventilation was adjusted according to blood gases. Arterial blood pH was kept between 7.35 and 7.45. Blood glucose was kept above 4 mM by intravenous infusion of isotonic glucose containing a potassium supplement (40 mM) to keep serum K^+ between 4 and 4.5 mM. A saline intravenous infusion of 400 mL·h^{-1} was maintained throughout the experiment. Body temperature was registered with a rectal probe and kept between 38 and 39 °C, which is the physiological range for pigs of this size.

The left anterior descending artery (LAD) was visualized with a 6 Fr left Judkin guiding catheter (Medtronic, MN, USA) introduced via the arterial sheath. An angioplasty balloon was placed just after the second diagonal branch. The balloon was inflated to total occlusion.

The pigs were divided into three groups as illustrated in Fig. 1. One group (n = 6) received a long ischemic stimulus of 45 minutes with total occlusion of the LAD (long ischemia). A second group (n = 9) underwent IPC for 10 minutes with total occlusion of the LAD followed by 30 minutes of reperfusion, repeated twice, prior to an ischemic stimulus of 45 minutes of total occlusion (long ischemia + IPC). The third group (n = 6) received a short ischemic stimulus of 20 minutes of total occlusion of the LAD (short ischemia). In all groups, total occlusion was confirmed angiographically immediately after each occlusion, before reperfusion and in case of DC conversion. Restored blood flow was documented angiographically after reperfusion. Ventricular fibrillation was treated with heart massage and DC conversion. No anti-arrhythmic drugs were given.

Thirty minutes after reperfusion, 410–681 MBq ^{99m}Tc-lactadherin, in average 0.18 mg (range 0.14–0.22 mg) of lactadherin, was injected intravenously. After 2 hours of reperfusion, the chest was opened by sternotomy and the heart was excised. Shortly before the excision, a tourniquet was placed around the LAD at the site of previous balloon occlusion. Evans blue (Alfa Aesar, Germany) 15 mL, 40% wt/vol, was injected into the left atrium for arterial distribution to the coronary vascular tree demarcating the AAR. After approximately 1 hour of freezing, the left ventricle (LV) was cut into 7-mm thick slices using a cutting machine (MultiSchneider, knife without serrations, Ritter E21, Germany). The slices were weighed and photographed. The IS was determined by 2,3,5,-triphenyltetrazodium chloride staining of the slices in a 1% wt/vol phosphate-buffered solution for about 15 minutes at 37 °C. The slices were re-photographed.

The areas of the LV and the IS as well as the AAR were quantified planimetrically using the image processing program ImageJ (wsr@nih.gov, National Institutes of Health, USA) and corrected for the weight of the individual slices. The observer variation was minimized using a computerized method.

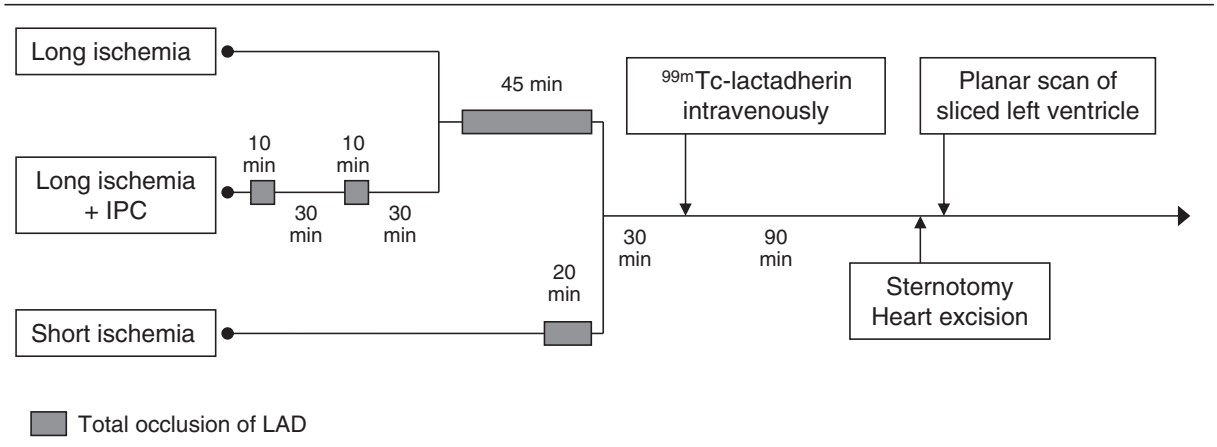

Fig. 1. An experimental study of the myocardial uptake of ^{99m}Tc-lactadherin administrated after different ischemic-reperfusion interventions.