Coronary microembolization during early reperfusion: infarct extension, but protection by ischaemic postconditioning

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Aims

Reperfusion injury following acute myocardial infarction impacts not only on the myocardium but also on the coronary microcirculation, and microembolization from the culprit lesion contributes to microvascular obstruction. Prior experimental studies have not accounted for microembolization in ischaemia/reperfusion injury and not considered microembolization as a confounder and target of protection by ischaemic postconditioning. We therefore investigated the impact of microembolization during reperfusion on infarct size and cardioprotection by postconditioning.

Methods and results

Anaesthetized, open-chest pigs were subjected to 90 min low-flow ischaemia. Immediate full reperfusion (n = 8) served as the control. Microembolization was induced by intracoronary infusion of 42 μm microspheres with the onset of reperfusion (n = 8). In a second step, postconditioning was induced by six cycles of 20s reperfusion/20s re-occlusion without (n = 8) and with superimposed microembolization (n = 8). Transmural blood flow and area at risk were determined by radioactive microspheres, infarct size by triphenyl tetrazolium chloride staining. Area at risk and transmural blood flow were not different between groups. Microembolization increased infarct size from 32 ± 3% of the area at risk to 47 ± 3% (P < 0.05). Embolizing particles were re-distributed away from the central infarcted area and accumulated in the infarct border, thus contributing to infarct extension. Postconditioning reduced infarct size without (21 ± 3%; P < 0.05 vs. immediate full reperfusion) and also with additional microembolization (26 ± 5%; P < 0.05 vs. immediate full reperfusion and microembolization); embolizing particles did not accumulate in the infarct border.

Conclusion

Microembolization at reperfusion augments infarct size, but postconditioning in the presence of microembolization still reduces infarct size and attenuates infarct expansion.

Keywords

Acute myocardial infarction • Cardioprotection • Coronary circulation • Microembolization • Reperfusion • Reperfusion injury

Introduction

Myocardial ischaemia/reperfusion impacts not only on the myocardium but also on the coronary microcirculation. The most severe form of coronary microvascular reperfusion injury is the ‘no-reflow’ phenomenon1–3 which is typically localized within the infarcted myocardium. Coronary microvascular obstruction4 results from microembolization of particulate debris,5 platelet aggregates,6 vasoconstriction in response to soluble factors which are released from the ruptured atherosclerotic lesion,7,8 and from injury and disruption of the microvascular endothelium.1,9,10 In both, primary percutaneous coronary interventions for reperfusion of an acute myocardial infarction and in elective coronary interventions, coronary microembolization is induced and contributes to the ultimate injury.5 Very recently, increased local release of platelet- and endothelium-derived microparticles into the coronary circulation has been identified in patients undergoing primary percutaneous coronary intervention for acute myocardial infarction and correlated

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Coronary microembolization during early reperfusion

Regional myocardial blood flow and infarct size

Radiolabelled microspheres (15 μm in diameter; 46Sc; NEN-DuPont, Boston, USA) were injected into the coronary perfusion circuit to determine transmural regional myocardial blood flow during ischaemia and the area at risk.22 Infarct size was determined by triphenyl tetrazolium chloride staining (TTC, Sigma-Aldrich Chemie GmbH, Munich, Germany). The amount of infarcted tissue is expressed as percent of the area at risk as determined by microspheres.

Coronary microembolization

Coronary microembolization was induced by slow infusion of 3000 microspheres (diameter 42 μm) per mL/min coronary inflow as determined at baseline. In preliminary experiments, this size and number of injected microspheres best reflected the pattern of microinfarcts that was previously observed in patients with unstable angina who died of sudden cardiac death and were subsequently autopsied.23,24

Microinfarcts

The perfusion system was clamped immediately before excision of the heart, and a blue dye (Patentblau, Guerbet, Sulzbach, Germany) was injected into the left atrium to stain remote myocardium. Transmural tissue samples were taken from the border zone of the unaffected region, and microinfarction was quantified in histological sections after haematoxylin/eosin staining. The visualization of microinfarcts was enhanced by phase-contrast microscopy (×100 magnification).

Mean analysed area per slide was 117 ± 16 mm². Infarct size is expressed as percent of the sum of all microinfarcted regions in relation to the total cross-sectional area of a specimen.

Experimental protocols

Protocol 1: immediate full reperfusion without and with coronary microembolization

Immediate full reperfusion (n = 8): Following baseline measurements of hemodynamics, coronary inflow was reduced to 10% of baseline and maintained constant. At 5 min ischaemia, measurements of haemodynamics and regional blood flow were performed. After 90 min ischaemia the myocardium was reperfused for 2 h.

Immediate full reperfusion and superimposed microembolization (n = 8): This protocol was identical to that of immediate full reperfusion, except that coronary microembolization was induced during the first minute of reperfusion. In five animals, reperfusion was extended to 4−5 h to facilitate the delineation of microinfarcts with histological quantification.

Methods

The experimental protocols were approved by the Bioethical Committee of the district of Düsseldorf.

Experimental preparation

The experimental setup has been described in detail previously.13 In brief, Göttinger minipigs (20−40 kg) were sedated by ketamine hydrochloride (1 g intramuscularly) and anaesthetized with thiopental (500 mg intravenously) and enflurane (1−1.5% in oxygen/nitrous oxide mixture). One common carotid artery was cannulated to measure arterial pressure and a jugular vein was cannulated for volume replacement. After a left lateral thoracotomy, the heart was exposed and a micromanometer (PS, Konigsberg Instr., Pasadena, CA, USA) introduced into the left ventricle. The other common carotid artery was cannulated with a polyethylene tube to supply arterial blood to an extracorporeal circuit, consisting of a roller pump, a windkessel to buffer pressure peaks induced by the phasicity of coronary blood flow, and a side port for the injection of radiolabelled microspheres and embolizing particles. Prior to filling of the extracorporeal circuit, the pigs were anticoagulated with 20 000 IU heparin sodium; additional doses of 10 000 IU were given at hourly intervals. The proximal left anterior descending coronary artery (LAD) was dissected over a distance of 1.5 cm, ligated, and cannulated, and the distal LAD was perfused from the extracorporeal circuit. Perfusion pressure was measured at a sidearm of the cannula tip with an external transducer (type 4-3271, Bell and Howell, Pasadena, CA, USA). To avoid any initial hypoperfusion under control conditions, the coronary arterial pressure was held above 75 mmHg by adjusting the rate of the perfusion pump. Regional myocardial ischaemia was induced by reducing the speed of the perfusion pump.

At the end of the protocol the heart was quickly excised and sectioned for postmortem analyses.

to indices of microvascular obstruction, such as TIMI frame count, myocardial blush grade, and electrocardiogram ST-segment resolution.11 To prevent coronary microembolization, thrombectomy is used in acute myocardial infarction, and protection devices are used in elective interventions, notably in saphenous vein grafts. In contrast, in most experimental studies healthy coronary arteries were subjected to occlusion and reperfusion, and the issue of coronary microembolization was largely neglected.12 We have therefore now compared ischaemia/reperfusion per se with ischaemia/reperfusion with superimposed coronary microembolization using an established large animal model of acute myocardial infarction and reperfusion.13

Coronary microembolization during early reperfusion not only contributes to reperfusion injury per se, but is also a confounder impairing the potential salvage of myocardium by cardioprotective manoeuvres.4,12,14–16 Ischaemic postconditioning attenuates reperfusion injury and reduces infarct size,17 and this protection is operative in all species tested so far,18 including humans.19

Most but not all studies in patients with acute myocardial infarction reported favourable results of ischaemic postconditioning on infarct size20 and some also on microvascular perfusion, as reflected by improved myocardial blush grade19 or TIMI frame count.21 We have therefore now, in a second step, also investigated coronary microembolization as a potential confounder and target of the protection by ischaemic postconditioning.

Distribution of embolizing particles

Tissue samples of ~2.5 g were taken from the central infarcted area, the infarct border zone, and from non-infarcted tissue within the area at risk, digested in 10 mol KOH, and filtrated using membranes with a pore size of 10 μm. Wet filter membranes were protected with round cover slips to prevent drying and loss of microspheres and then placed on glass slides. At ×100 magnification the number of 42 μm particles on the filter was counted. The number of embolizing particles per tissue sample was normalized to that in the non-infarcted area at risk.

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Immediate full reperfusion and ischemic postconditioning without and with coronary microembolization

Table 1: Systemic hemodynamics

<table>
<thead>
<tr>
<th>Time</th>
<th>HR (1/min)</th>
<th>LVPmax (mmHg)</th>
<th>dP/dtmax (mmHg/s)</th>
<th>CAPMean (mmHg)</th>
<th>CBFMean (μl/min)</th>
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<tr>
<td>IFR</td>
<td>115 ± 45</td>
<td>159 ± 61*</td>
<td>128 ± 83*</td>
<td>148 ± 74*</td>
<td>40 ± 4*</td>
</tr>
<tr>
<td>+</td>
<td>103 ± 5*</td>
<td>97 ± 3*</td>
<td>104 ± 6*</td>
<td>107 ± 5*</td>
<td>39 ± 3*</td>
</tr>
<tr>
<td>IFR+Co</td>
<td>99 ± 5*</td>
<td>103 ± 5*</td>
<td>112 ± 8*</td>
<td>116 ± 6*</td>
<td>34 ± 3*</td>
</tr>
<tr>
<td>+Co</td>
<td>100 ± 5*</td>
<td>97 ± 3*</td>
<td>104 ± 6*</td>
<td>107 ± 5*</td>
<td>39 ± 3*</td>
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<tr>
<td>IFR+Co+ME</td>
<td>99 ± 5*</td>
<td>103 ± 5*</td>
<td>112 ± 8*</td>
<td>116 ± 6*</td>
<td>34 ± 3*</td>
</tr>
<tr>
<td>+Co+ME</td>
<td>100 ± 5*</td>
<td>97 ± 3*</td>
<td>104 ± 6*</td>
<td>107 ± 5*</td>
<td>39 ± 3*</td>
</tr>
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*P < 0.05 vs. baseline; two-way ANOVA for repeated measures and Fisher's least significant difference post hoc tests.
were harvested at 6 h after microembolization. Microinfarcts were also quantified.

**Statistics**

Data are mean ± SEM. All statistical analyses were performed with SigmaStat for Windows Version 2.03 (formerly SPSS Inc., Chicago, USA). Haemodynamics and distribution of embolizing particles were analysed by two-way analysis of variance (ANOVA) for repeated measures. Areas at risk and infarct sizes were analysed by one-way ANOVA. When a significant difference was detected, individual mean values were compared by post hoc tests (Fisher’s least significant difference test, two-sided). Differences were considered significant at the level of $P < 0.05$.

**Results**

Systemic haemodynamics were not different between groups (Table 1). The area at risk and transmural blood flow during ischaemia were comparable between groups (Figures 1 and 2). 

**Protocol 1: immediate full reperfusion without and with coronary microembolization**

Coronary microembolization increased infarct size from $32 \pm 3\%$ of the area at risk with immediate full reperfusion to $47 \pm 3\%$ with immediate full reperfusion and superimposed microembolization ($P < 0.05$; Figure 1A). Microinfarcts in the TTC-positive area at

Figure 1 Protocol 1: ischaemia/reperfusion without and with coronary microembolization. (A) Transmural blood flow (TMBF), area at risk (AAR), and infarct size. Coronary microembolization augments embolizing particle size. IFR, immediate full reperfusion; IFR+ME, immediate full reperfusion and coronary microembolization. (B) Spatial distribution of embolizing particles in the area at risk. The distribution of particles is displayed for each individual animal subjected to IFR+ME. Grey bars (the bar width corresponds to infarct size) indicate samples from infarcted tissue. Samples are displayed from septal areas (SE, left) to samples from the anterior wall (AW, right). Open diamond symbols indicate tissue samples located within the infarct border zone from animals undergoing IFR+ME. (C) Statistical analysis of spatial distribution of embolizing particles. Data from corresponding non-infarcted and border zones from septal and anterior wall regions of the AAR were combined. N, non-infarcted AAR; Nb, non-infarcted AAR at the border to infarcted tissue; Ib, infarcted AAR at the border to non-infarcted tissue; I, central infarcted area; *$P < 0.05$ vs. N)
risk amounted to 5.5 ± 1.7% of the analysed total cross-sectional area (0.228 ± 0.046 mm² area of individual microinfarcts). The embolizing particles were not homogeneously distributed within the area at risk (Figure 1B). With immediate full reperfusion, a consistent pattern emerged, with decreased particles per myocardial tissue mass in the central infarct and a reciprocal increase in the infarct border (Figure 1C).

Protocol 2: immediate full reperfusion and ischaemic postconditioning without and with coronary microembolization

Ischaemic postconditioning reduced infarct size (21 ± 3%; P < 0.05 vs. immediate full reperfusion in protocol 1), also when associated with coronary microembolization (26 ± 5%; P < 0.05 vs. immediate full reperfusion and superimposed microembolization in protocol 1; Figure 2A). With postconditioning, microinfarcts in the TTC-positive area at risk amounted to 5.6 ± 1.9% of the analyzed total cross-sectional area (0.202 ± 0.016 mm² area of individual microinfarcts). The inhomogeneous distribution pattern of embolizing particles seen with immediate full reperfusion was not observed in animals subjected to ischaemic postconditioning (Figure 2B). Here, the lowest numbers of particles were also found in infarcted regions, but there was no consistent overshoot in the infarct border zone (Figure 2C).

Embolizing particles were homogeneously distributed during normoperfusion (Figure 3A and B).

Discussion

The present study is to our knowledge the first one to systematically evaluate the impact of coronary microembolization during reperfusion on infarct size and on the protection by ischaemic postconditioning.

Our experimental approach simulates the release of atherothrombotic debris during primary percutaneous coronary
intervention in patients with acute myocardial ischaemia.\textsuperscript{4,5,11} However, the impact of periprocedural microembolization on infarct size cannot be readily estimated from patient studies due to the large heterogeneity in the patient population (duration of ischaemia, collaterals, comorbidities, and medication), the lack of a true control group and the unpredictable amount of coronary microembolization. Therefore, we have used our established model in pigs,\textsuperscript{27,28} in which factors that influence infarct size, such as the duration of ischaemia and systemic haemodynamics can be tightly controlled to isolate the genuine effect of microembolization on infarct size.

The group sizes in protocols 1 and 2 were estimated from our previous experiments. The small sample size with microembolization at normoperfusion was justified by the fact that none of the animals showed the typical particle distribution pattern observed in all the pigs subjected to coronary microembolization at reperfusion, but rather the expected flat distribution. The reduction of coronary blood flow to 10% of baseline during the sustained ischaemia in this model is a realistic estimate of the residual blood flow in the myocardium at risk from patients with acute myocardial infarction, as determined by a semiquantitative index derived from \textsuperscript{99m}Tc-sestamibi tomography.\textsuperscript{29}

The amount of injected embolizing particles was individually adjusted in each animal to account for differences in the size of the area at risk.\textsuperscript{30} The amount of microinfarction as determined by the histological analysis of macroscopically non-infarcted tissue revealed microinfections with a similar total amount as observed previously in this model.\textsuperscript{30,31} Surprisingly, microembolization increased infarct size significantly by 15% and thus largely above what would be expected with the amount of microinfection (3.4% with immediate full reperfusion, 4% with immediate full reperfusion and ischaemic postconditioning). Although different methodology was used to determine macroscopic infarct size (TTC) and aggregate microinfection (histology), such different methodologies can not explain the large and unexpected increase in infarct size above what could be expected from microinfarction. What makes microembolization at reperfusion so deleterious? We observed an uneven distribution of embolizing particles per tissue mass, with a shift from the core area of infarction to the infarct border zone. This inhomogeneity of microsphere distribution was not observed during normoperfusion and can therefore not be attributed to the pump-perfusion of the coronary artery. The redistribution of embolizing particles following ischaemia/reperfusion probably resulted from a developing no-reflow phenomenon\textsuperscript{1,32} which can affect about 10% of the area at risk already at 2 min after the onset of reperfusion.\textsuperscript{9} Such rapidly developing no-reflow areas can redistribute the embolizing particles at early reperfusion and thus induce a lateral expansion of the infarcted area. The increased amount of embolizing particles in the infarct border still cannot fully account for the observed augmentation of infarct size. Therefore, the vulnerability of the border zone of the freshly reperfused myocardium most likely also contributes. The potential damage by coronary microembolization in a clinical study is probably still largely underestimated by our use of inert particles\textsuperscript{31} whereas microemboli in the clinical setting of reperfused myocardial infarction will contain debris from the ruptured atherosclerotic lesion with superimposed platelets, leukocytes, and thrombotic material. Also, soluble vasoconstrictor, thrombogenic and inflammatory substances are released in clinical scenarios of coronary microembolization and contribute to microvascular impairment.\textsuperscript{7,8,33}

In contrast, in pigs subjected to ischaemic postconditioning in the presence of microembolization, the observed increase in final infarct size was only 5% and thus close to the expected range. Correspondingly, the distribution of embolizing particles was more even, possibly reflecting attenuation of the no-reflow phenomenon and related to a reduction of oedema with immediate full reperfusion and ischaemic postconditioning.\textsuperscript{17,34} Obviously,
postconditioning did not reduce the amount of microinfarction, in line with the notion that for postconditioning to protect some reperfusion is mandatory. However, postconditioning largely attenuated the no-reflow zone and thus the deleterious redistribution of embolizing particles into the infarct border. Thus, postconditioning both reduced infarct size and the augmentation of infarct size by microembolization. Accordingly, the benefit from postconditioning was even larger in the presence of coronary microembolization (45% relative reduction in infarct size from that by ischaemia/reperfusion without postconditioning) than in its absence (34%) (Figure 2A). Our observations fit very well to the notion of Whittaker and Przyklenk that it is the border zone which is at the cutting ‘edge’ to experience more damage, in this case by microembolization, or protection, in this case by ischaemic postconditioning. Ischaemic postconditioning did not improve the haemodynamic recovery during reperfusion; apparently, the haemodynamics reflect not only the amount of infarcted tissue but also a superimposed stunning which is not reversible within the 2 h reperfusion period. In the present study, potentially involved signalling molecules of cardioprotection, i.e. increased tumour necrosis factor-α secondary to coronary microembolization and signal transducer and activator of transcription-3 activation by ischaemic postconditioning could not be analysed. The precise localisation of the infarct within the area at risk and in particular the rather small infarct border zone becomes apparent only with postmortem TTC staining. Thus, it is technically impossible to harvest fresh tissue samples during the experiment for analysis of signalling molecules.

In conclusion, coronary microembolization substantially contributes to myocardial ischaemia/reperfusion injury and should be considered in more realistic experimental studies on cardioprotection. Ischaemic postconditioning exerts greater protection in the presence than in the absence of coronary microembolization because it protects not only from cardiomyocyte injury but also from microvascular impairment.

Conflict of interest: none declared.

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References


