Evaluation of Cardioprotective Effects of Recombinant Soluble P-Selectin Glycoprotein Ligand-Immunoglobulin in Myocardial Ischemia-Reperfusion Injury by Real-Time Myocardial Contrast Echocardiography

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OBJECTIVES
The purpose was to examine the cardioprotective effects of recombinant P-selectin glycoprotein ligand-immunoglobulin (rPSGL-Ig) in ischemia-reperfusion injury by real-time myocardial contrast echocardiography (MCE).

BACKGROUND
P-selectin mediates leukocyte recruitment into areas of inflammation.

METHODS
Sixteen pigs underwent 45 min of left anterior descending coronary artery occlusion followed by reperfusion and received rPSGL-Ig or vehicle. To assess changes in myocardial perfusion (Aβ), MCE was performed.

RESULTS
After 120 min of reperfusion, Aβ in the risk area was higher (0.84 ± 0.15 dB/s vs. 0.28 ± 0.1 dB/s, p < 0.0001), and the infarct size was lower (30.3 ± 12% vs. 57 ± 14%, p = 0.002) in the rPSGL-Ig group compared with the vehicle group.

CONCLUSIONS
Recombinant PSGL-Ig improved postischemic reflow accurately detected by real-time MCE. (J Am Coll Cardiol 2004;44:887–91) © 2004 by the American College of Cardiology Foundation

Microcirculatory reflow after myocardial infarction is intimately related to functional recovery (1). Leukocytes can induce microvascular damage in the reperfused myocardium, known as “ischemia-reperfusion injury” (2,3). P-selectin glycoprotein ligand-1 (PSGL-1) is the physiologic ligand for endothelial P-selectin expressed on leukocytes, as well as a key molecule responsible for recruitment of neutrophils into areas of inflammation (4,5). Recombinant PSGL-Ig, a recombinant immunoglobulin form of PSGL-1, acts as a competitive inhibitor for P-selectin (6,7). Therefore, immunoneutralization of P-selectin should reduce the extent of leukocyte-mediated tissue damage. The purpose of this study was to investigate whether real-time myocardial contrast echocardiography (MCE) can be used to evaluate microvascular reflow and the cardioprotective effects of rPSGL-Ig.

METHODS
Experimental protocol. Sixteen farm pigs (20 to 25 kg) received 2,500 IU heparin and 500 mg acetylsalicylic acid and underwent left anterior descending coronary artery (LAD) occlusion below the first branch of the LAD for 45 min, followed by 120-min reperfusion. A dose of 1 mg/kg rPSGL-Ig (6) or vehicle was given during ischemia.

Myocardial contrast echocardiography. Real-time MCE was performed serially with an HDI5000 (ATL, Bothell, Washington) during intravenous infusion of SonoVue (60 ml/h; Bracco, Milan, Italy) at a low mechanical index of 0.09 and analyzed using HDI software (ATL). The risk area was identified as the area of absent myocardial perfusion (Aβ) in the risk area was higher (0.84 ± 0.15 dB/s vs. 0.28 ± 0.1 dB/s, p < 0.0001), and the infarct size was lower (30.3 ± 12% vs. 57 ± 14%, p = 0.002) in the rPSGL-Ig group compared with the vehicle group.

Histologic examinations and myocardial blood flow (MBF). The LAD was reoccluded at the end of the experiment, and 1 mg/kg monastral blue dye (Sigma, St. Louis, Missouri) was injected into the left atrium to outline the area at risk. The heart was cut into 1-cm-thick slices. From the myocardial slice corresponding to the MCE imaging plane transmural myocardial tissue, samples were excised from the central portion of the risk area, the ischemic border zone (unstained by blue dye), and the control area, divided, and processed either for analysis of capillary density, as described (8), or measurements of MBF.

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Abbreviations and Acronyms

A × β = myocardial perfusion
FAS = fractional area shortening
LAD = left anterior descending coronary artery
LV = left ventricle
MBF = myocardial blood flow
MCE = myocardial contrast echocardiography
PSGL-1 = P-selectin glycoprotein ligand-1
rPSGL-Ig = recombinant P-selectin glycoprotein ligand-immunoglobulin

same time points as MCE imaging (except 30 min reperfusion), as described previously (10).

Myocardial slices were immersed in 1.3% triphenyltetrazolium chloride (Sigma), which stains viable myocardium red. The infarct and risk areas in each slice were determined by planimetry. The risk area by histology was expressed as the percentage of the total left ventricle (LV) (equal to sum of all slices), and the infarct size was expressed as the percentage of risk area.

Statistical analysis. Data are expressed as the mean value ± SD, and statistical significance was set at p < 0.05. Correlations between continuous variables were performed by linear regression analysis. The Student t test, paired or unpaired, as appropriate, was used for group-to-group comparisons.

RESULTS

Hemodynamics and histologic analysis. Mean arterial pressure and FAS decreased during reperfusion but were higher in the rPSGL-Ig group after 120-min reperfusion (Table 1). Left ventricular weight was similar between the rPSGL-Ig and vehicle groups (66 ± 16 g vs. 61 ± 13 g, p = NS), as well as the risk area (% total LV) by monastral blue staining (19.4 ± 4% vs. 22.3 ± 6%, p = NS). The infarct size to risk area by TTC staining was 57 ± 14% and decreased to 30.3 ± 12% (p = 0.002) in the rPSGL-Ig group. Microscopic analysis demonstrated extensive myocardial injury in the risk area, but the numerical capillary density was higher in the rPSGL-Ig group in the central portion of the risk area, as described previously (10). Myocardial blood flow (MBF, microsphere-derived) was assessed in the central portion and ischemic border of the risk area and nonischemic control area. Data are presented as the mean value ± SD.

FAS = fractional area shortening; HR = heart rate; MAP = mean arterial pressure.

Table 1. Myocardial Function and Perfusion

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Ischemia</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>82 ± 10</td>
<td>75 ± 9</td>
<td>80 ± 11</td>
<td>78 ± 9</td>
<td>74 ± 6</td>
<td>70 ± 7*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>71 ± 15</td>
<td>110 ± 20*</td>
<td>106 ± 9*</td>
<td>115 ± 11*</td>
<td>108 ± 9*</td>
<td>100 ± 18*</td>
</tr>
<tr>
<td>FAS (%)</td>
<td>55 ± 8</td>
<td>34 ± 9*</td>
<td>51 ± 7</td>
<td>48 ± 9</td>
<td>41 ± 7*</td>
<td>32 ± 6*</td>
</tr>
<tr>
<td>MBF (ml/g/min)</td>
<td>1.29 ± 0.4</td>
<td>0.03 ± 0.01*</td>
<td>1.69 ± 1.2</td>
<td>—</td>
<td>1.32 ± 0.9</td>
<td>0.85 ± 0.4*</td>
</tr>
<tr>
<td>Ischemic border</td>
<td>1.23 ± 0.3</td>
<td>0.04 ± 0.01*</td>
<td>1.39 ± 1.3</td>
<td>—</td>
<td>1.72 ± 0.8</td>
<td>1.20 ± 0.3</td>
</tr>
<tr>
<td>Control area</td>
<td>1.35 ± 0.3</td>
<td>1.40 ± 0.4</td>
<td>1.43 ± 0.2</td>
<td>—</td>
<td>1.39 ± 0.4*</td>
<td>1.21 ± 0.4</td>
</tr>
<tr>
<td>rPSGL-Ig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>79 ± 8</td>
<td>72 ± 8*</td>
<td>83 ± 10</td>
<td>77 ± 11</td>
<td>84 ± 8</td>
<td>81 ± 9*</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>78 ± 9</td>
<td>105 ± 14*</td>
<td>108 ± 10*</td>
<td>100 ± 12</td>
<td>102 ± 15</td>
<td>95 ± 13</td>
</tr>
<tr>
<td>FAS (%)</td>
<td>51 ± 6</td>
<td>38 ± 10*</td>
<td>54 ± 7</td>
<td>50 ± 10</td>
<td>49 ± 7</td>
<td>44 ± 8†</td>
</tr>
<tr>
<td>MBF (ml/g/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central risk area</td>
<td>1.41 ± 0.7</td>
<td>0.10 ± 0.1*</td>
<td>2.71 ± 1.2*</td>
<td>—</td>
<td>2.45 ± 0.8†</td>
<td>1.65 ± 0.5†</td>
</tr>
<tr>
<td>Ischemic border</td>
<td>1.53 ± 0.7</td>
<td>0.12 ± 0.1*</td>
<td>2.89 ± 1.4*</td>
<td>—</td>
<td>2.66 ± 0.7</td>
<td>1.86 ± 0.4‡</td>
</tr>
<tr>
<td>Control area</td>
<td>1.43 ± 0.3</td>
<td>1.45 ± 0.8</td>
<td>1.48 ± 0.5</td>
<td>—</td>
<td>1.56 ± 0.6</td>
<td>1.38 ± 0.5</td>
</tr>
</tbody>
</table>

p < 0.05 for comparisons between baseline and same group or between vehicle and recombinant P-selectin glycoprotein ligand-immunoglobulin (rPSGL-Ig). Myocardial blood flow (MBF, microsphere-derived) was assessed in the central portion and ischemic border of the risk area and nonischemic control area. Data are presented as the mean value ± SD.

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0.001), and A correlated with capillary density ($r = 0.8$, $p = 0.007$).

Repeated measurements of $A \times \beta$ showed a good reproducibility ($r = 0.93$, $p < 0.0001$), with a mean $\pm 2$ SD of $0.013 \pm 0.38$ dB/s. The risk area was not significantly different when assessed 3 or 10 heart cycles after flash, which demonstrates low collateral flow in pigs.

**DISCUSSION**

The present study outlines the pathophysiologic implication of P-selectin in the development of ischemia-reperfusion injury. Immunoneutralization of P-selectin by rPSGL-Ig reduced the infarct size and preserved microvascular integrity accurately detected by real-time MCE.

**Assessment of ischemia-reperfusion injury by MCE.** Triggered MCE techniques have been used extensively in acute coronary syndromes and shifted the interest from infarct-related artery patency to microvascular integrity (1–3,8). However, practical considerations have limited its broader clinical use. Real-time MCE allows simultaneous evaluation of myocardial perfusion and contractility and obviates the need for background subtraction, but little information is available on the detection of postischemic microvascular dysfunction. In this study, myocardial perfusion could be easily visualized and quantified by real-time MCE. Furthermore, MCE confirmed a fully reperfused risk area characterized by absent perfusion defects and hyperemic reflow. During follow-up, the MBF index $A \times \beta$ decreased with reappearance of a marked perfusion defect. The infarct size and MBF by MCE corresponded well with standard techniques. These findings are in agreement with previous reports based on triggered and real-time techniques (11,12).

Kunichika et al. (12) recently demonstrated the usefulness of real-time MCE to assess the cardioprotective effects of glycoprotein IIb/IIIa inhibition in a canine model of 3-h ischemia. In contrast to our model, no significant changes in perfusion during reperfusion were reported, most likely reflecting extensive irreversible injured myocardium occurring during ischemia, as well as the inability to reperfuse the myocardium. The duration of ischemia was shorter in our study (45 min), resulting initially in hyperemic reflow with a gradual decrease in myocardial perfusion patterns A and $\beta$.

This supports the existence of ischemia-reperfusion injury
characterized by serial changes in the postischemic microcirculation.

**Cardioprotective effects of rPSGL-Ig.** In the present study, we used rPSGL-Ig, a recombinant immunoglobulin form of PSGL-1, which has been mutated to reduce both complement activation and Fc receptor binding with a half-life of ~11 days in pigs (4–6). As a result, P-selectin blockade by rPSGL-Ig administrated at the time of reperfusion and the infarct size to risk area of the corresponding myocardial slice by TTC staining (bottom) in the rPSGL-Ig (circles) and vehicle (squares) groups.

Figure 2. Time course in the perfusion defect size to risk area by myocardial contrast echocardiography (MCE) (top). *p < 0.05 for comparisons between vehicle (solid lines) and recombinant P-selectin glycoprotein ligand-immunoglobulin (rPSGL-Ig) (dashed lines). Correlation between the perfusion defect size to risk area by MCE at 120-min reperfusion and the infarct size to risk area of the corresponding myocardial slice by TTC staining (bottom) in the rPSGL-Ig (circles) and vehicle (squares) groups.

Figure 3. Quantitative myocardial contrast echocardiography parameters A (top), β (middle), and A × β (bottom) in the left anterior descending coronary artery (LAD) territories at baseline (bas), during LAD occlusion (occ), and reperfusion. The decrease in indexes of myocardial perfusion during reperfusion was lower in the recombinant P-selectin glycoprotein ligand-immunoglobulin (rPSGL-Ig) group (dashed lines) than in the vehicle group (solid lines). *p < 0.05.
which has potential implications, as leukocyte-platelet aggregates contribute to microvascular obstruction. Indeed, P-selectin blockade reduced platelet deposition on injured arterial surfaces (13) and enhanced thrombolysis (6) without an increase in bleeding rate.

**Clinical implications.** Our study provides strong evidence of the detrimental effects of postischemic reperfusion and suggests a potential role of P-selectin in the pathogenesis of ischemia-reperfusion injury. However, it is not easy to speculate whether rPSGL-Ig may provide a beneficial adjunct to standard reperfusion strategies. The selectin antagonist CY-1503 reduced the extent of reperfusion lung injury (14), but rPSGL-Ig, currently studied in phase I/II clinical trials, failed to improve thrombolysis in patients with myocardial infarction (15). However, soluble P-selectin and intercellular adhesion molecule-1 concentrations were consistently elevated after percutaneous transluminal coronary angioplasty but not after thrombolysis with tissue-type plasminogen activator (16). Additional studies are needed to further elucidate this clearly exciting approach to preserve microvascular integrity. Because real-time MCE has been improved both qualitatively and quantitatively, it may become invaluable in the diagnosis and management of acute ischemic syndromes.

**REFERENCES**