Role of Platelet-Activating Factor on Extravascular Lung Water after Coronary Reperfusion in Dogs

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Abstract: Platelet-activating factor (PAF), one of the harmful substances released after coronary reperfusion, has been reported to increase pulmonary vascular permeability and induce pulmonary edema. In this study, we sought to examine the possible role of PAF in the genesis of pulmonary edema after coronary reperfusion. Extravascular lung water (EVLW) was measured by the thermal-dye double indicator dilution method during coronary ligation and after reperfusion in situ in dogs. The proximal left anterior descending coronary artery was occluded for 15 min and reperfused in 5 dogs (group 1), while five other dogs (group 2) were treated with PAF-antagonist (TCV-309, 1 mg/kg) before coronary artery occlusion. EVLW and hemodynamic indices were measured at baseline, 15 min of coronary occlusion, and 15 and 30 min after coronary reperfusion. EVLW increased at 15 min of coronary occlusion in both groups, but there was no significant difference between the two groups (6.4 to 10.3 ml/kg and 5.4 to 7.1 ml/kg in groups 1 and 2, respectively). After coronary reperfusion, EVLW increased further in group 1 (6.4 to 16.5 ml/kg, p < 0.01), but no further increase was observed in group 2 at 30 min after coronary reperfusion. There were no significant differences in hemodynamic indices between the two groups throughout the test. Thus, PAF-antagonist attenuated the increase in EVLW after coronary reperfusion independent of hemodynamic indices, and hence, PAF may play an important role in the genesis of pulmonary edema caused by coronary reperfusion. [Japanese Journal of Physiology, 48, 157–161, 1998]

Key words: extravascular lung water, pulmonary edema, reperfusion, dog.

Although one of the therapies for acute myocardial infarction (MI) is coronary reperfusion, there has been concern that reperfusion in itself might have deleterious effects on the myocardium [1]. Reperfusion injury is caused by calcium overload [2, 3] or by certain harmful substances such as superoxide free radicals, platelet-activating factor (PAF), and prostaglandin metabolites, which also increase pulmonary vascular permeability and induce pulmonary edema [4, 5]. However, few studies have focused on pulmonary edema after coronary reperfusion. In this study, we hypothesized that extravascular lung water (EVLW) may increase after coronary reperfusion, and PAF may have a role in aggravating pulmonary edema after coronary reperfusion. To test this hypothesis, we studied the effect of a specific PAF antagonist, TCV-309, on the accumulation of EVLW after coronary reperfusion in dogs.

METHODS

General preparation. Ten mongrel dogs weighing between 10 and 20 (16 ± 4) kg were anesthetized with intravenous pentobarbital (25 mg/kg) and supplemented as necessary during the experiment. The dogs were ventilated through a cuffed orotracheal tube by a Harvard animal respirator with room air at a tidal volume of 15 ml/kg and a rate of 13 to 15 breaths/min. The dogs’ lungs were inflated every 15 min to minimize microatelectasis.
A 7F Swan-Ganz catheter was placed into a pulmonary artery from a femoral vein, and the position of the catheter was determined from the pressure recordings. A 5F lung water catheter (Edwards Laboratory, Model 96-020) was advanced into the descending aorta from the femoral artery. A pacing wire was placed at the right atrium and constantly paced at a rate of 120 beats/min (Fig. 1).

**Measurements.** EVLW measurements were made by injecting 10 ml of cold (0°C) saline containing 5 mg of indocyanine green into the right atrium through the Swan-Ganz catheter. Simultaneously, blood was withdrawn by a syringe pump through a densitometer cuvette (Waters, DC-410) attached to the femoral lung water catheter. A dye signal proportional to concentration was generated in the densitometer and a thermal signal proportional to temperature was generated from the thermistor at the end of the lung water computer. Both signals were fed into a computer (Edward Laboratory, Model 9310) that simultaneously determined the mean transit time of the two indicators and cardiac output. EVLW was derived as follows: EVLW = cardiac output × (thermal mean transit time minus dye mean transit time) [3, 4, 6–8].

Blood pressure was measured by a transducer connected to the femoral arterial catheter, and pulmonary arterial pressure was obtained from a transducer connected to the Swan-Ganz catheter. Measurements were performed during a 10 s breath-hold at end of expiration. Limb leads and epicardial leads in the electrocardiogram were continuously monitored to evaluate heart rate and myocardial ischemia throughout the experiment.

**Experimental protocol.** Five dogs (group 1) received a vehicle injection (10 ml, 0.9% saline) and five dogs (group 2) received an injection of TCV-309 (1 mg/kg) in 0.9% saline (10 ml) 10 min before coronary ligation. A suture was placed around the left anterior descending artery above the first major diagonal branch. Myocardial ischemia was confirmed by ST segment changes in the epicardial electrocardiogram and abnormal regional contraction in the distribution of the ligated vessel. Coronary reperfusion was confirmed by normalization of the ST segment and regional contraction. Regional contraction was visually observed by three investigators. EVLW and hemodynamic indices were measured at baseline, 15 min of coronary ligation, and 15 and 30 min after reperfusion.

All experimental studies were performed according to the animal use guidelines set by the National Institutes of Health and the American Physiological Society.

**Test drug.** TCV-309 (3-bromo-5-([N-phenyl-N-(2-[(1,2,3,4-tetrahydro-2-isoquinolyl)carbonyl]oxy)ethyl]carbamonyl)ethyl]carbamoyl]-1-propylpyridium nitrate) was developed and supplied by Takeda Chemical Industries, Osaka, Japan.

**Statistical analyses.** Values are expressed as mean±SEM. The effects of PAF at each phase were analyzed using ANOVA with a repeated measures design (SuperANOVA v.1.1, Abacus Concepts, Berkeley, CA, USA). When significant differences between the two groups were detected by ANOVA, non-paired t-tests with Bonferroni’s correction for multiple comparisons were performed to determine which individual differences were statistically significant. An effect with p<0.05 was considered statistically significant.
Role of PAF in the Genesis of Pulmonary Edema

Table 1. Changes in hemodynamic indices.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ligation 15 min</th>
<th>Reperfusion 15 min</th>
<th>Reperfusion 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1 (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI (ml/min/kg)</td>
<td>162±13</td>
<td>129±12</td>
<td>110±25</td>
<td>110±23</td>
</tr>
<tr>
<td>PCW (mmHg)</td>
<td>9±1</td>
<td>11±1*</td>
<td>9±2</td>
<td>9±1</td>
</tr>
<tr>
<td>mPA (mmHg)</td>
<td>17±2</td>
<td>18±1</td>
<td>13±1</td>
<td>16±1</td>
</tr>
<tr>
<td>mBP (mmHg)</td>
<td>83±8</td>
<td>77±6</td>
<td>80±3</td>
<td>79±4</td>
</tr>
<tr>
<td><strong>Group 2 (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI (ml/min/kg)</td>
<td>139±4</td>
<td>120±10</td>
<td>122±10</td>
<td>129±15</td>
</tr>
<tr>
<td>PCW (mmHg)</td>
<td>11±1</td>
<td>14±2*</td>
<td>12±2</td>
<td>10±2</td>
</tr>
<tr>
<td>mPA (mmHg)</td>
<td>17±2</td>
<td>21±4</td>
<td>18±3</td>
<td>16±2</td>
</tr>
<tr>
<td>mBP (mmHg)</td>
<td>83±4</td>
<td>81±5</td>
<td>82±6</td>
<td>84±6</td>
</tr>
</tbody>
</table>

Mean±SEM. CI, cardiac index; PCW, pulmonary capillary wedge pressure; mPA, mean pulmonary artery pressure; mBP, mean blood pressure. * p<0.05 vs. baseline.

RESULTS

Changes in hemodynamic indices during coronary ligation and after reperfusion

The hemodynamic data are presented in Table 1. CI (cardiac index) were decreased and PCW (pulmonary capillary wedge pressure) were increased during coronary ligation in both groups. However, repeated measured ANOVA revealed that there were no significant differences of these changes between the two groups in the hemodynamic indices during coronary ligation and after reperfusion.

Changes in EVLW during coronary ligation and reperfusion

The changes in EVLW during coronary ligation and after reperfusion are shown in Fig. 2. There were significant differences in the change of EVLW between the two groups as measured by repeated ANOVA. There was no significant difference in EVLW between the two groups at baseline by non-paired t-tests. As a result, the amount of increase in EVLW was larger in group 1 than in group 2 at 30 min after reperfusion (6.4 to 16.5 ml/kg and 5.4 to 7.6 ml/kg, respectively).

DISCUSSION

One of the most important developments in the treatment of myocardial infarction (MI) is establishing early reperfusion of the ischemic heart muscle in the early hours of MI. Early reperfusion is reported to improve hemodynamic indices and decrease infarct size [9–13]. Despite this observation, there has been concern that the act of reperfusion in itself might have some deleterious effects on the myocardium [1]. The cause of reperfusion injury includes calcium overload or due to certain harmful substances, which include superoxide free radicals, eicosanoids and PAF [4, 5, 14–16]. Although these substances are reported to cause myocardial damage, few studies have focused on the effect of these substances on the genesis of pulmonary edema after coronary reperfusion. Because the normalization of ventricular wall motion is observed within 20 min after coronary occlusion [17], occlusion time in this study was set at 15 min. As a result, we observed a further increase in EVLW after coronary reperfusion in dogs. Several investigators found an excellent correlation between EVLW measured by the thermal-dye method and by gravimetric estimates of lung water content in experimental animals [6–8]. In our laboratory, we found a close correlation between these two techniques in dogs [18]. Thus, the thermal-dye method is an accurate method for determining the change in EVLW accumulation. However, there are still some problems. The accuracy of this method is limited by the presence of markedly
uneven lung perfusion such as in pulmonary emboli, a low cardiac output state and pulmonary hypertension [8, 16, 19]. Nevertheless, we believe that it is an accurate method to investigate the effect of PAF on pulmonary edema in this study, because the changes of hemodynamic status were not difference between the two groups.

PAF is a lipid mediator with diverse biological actions such as the activation of platelets [20]. PAF increases vascular permeability and causes several blood components to leak out of the vasculature, which leads to an increase in myocardial tissue pressure and edema [20]. PAF also activates neutrophils and stimulates them to produce various substances, including leukotrienes and superoxide free radicals [21], which cause myocardial cell injury. Several PAF antagonists (SR163-441, SR163-675, WEB2086) have been reported to attenuate or block endotoxin-induced pulmonary edema [22–24]. PAF antagonists have a beneficial effect in reducing myocardial necrosis, independent of hemodynamics [25]. TCV-309, used in this study, has the pharmacological profile of a novel specific PAF antagonist (competitive inhibitor of PAF receptor [26, 27]), is more potent than CV-3988 and CV-6209, and is as potent as WEB 2086 in the inhibition of PAF-induced platelet aggregation [28]. TCV-309 protected mice from death due to anaphylactic shock induced by PAF, and also rats from death induced by endotoxin [23, 25]. Several investigators reported that PAF causes pulmonary vasoconstriction in normally oxygenated animals [29–31]. However, in low doses (10–100 µg/min), PAF did not change the pulmonary circulation [32]. Montrucchio et al. showed that PAF is released in significant but low doses (10.9±12.8µg/min) after coronary reperfusion [33]. In this study, pulmonary artery pressure was not elevated after reperfusion in either group.

Recently, Taniguchi et al. reported that PAF antagonist (CV-6209) attenuated an increase of EVLW during coronary ligation in dogs without increasing pulmonary capillary wedge pressure, and suggested that PAF may play an important role in the genesis of pulmonary edema during ischemia [4]. In this study, we examined the effect of a specific PAF antagonist, TCV-309, on the attenuation of pulmonary edema during reperfusion. To minimize the effect of TCV-309 on ischemia, we reduced the ischemic duration to 15 min; one-third of the ischemic duration employed in our previous study [4]. Indeed, there was no significant difference in EVLW between the two groups at the end of ischemia.

In this study, EVLW increased during coronary ligation in both groups, and was associated with increased pulmonary capillary wedge pressure. Although pulmonary arterial and pulmonary capillary wedge pressures returned to control levels after coronary reperfusion in both groups, EVLW increased further in group 1. However, the increase in EVLW after coronary reperfusion was inhibited by pretreatment with TCV-309, indicating that TCV-309 can prevent the accumulation of EVLW caused by coronary reperfusion.

The limitations of our study should be addressed: PAF and other chemical mediators including oxidized phospholipids can participate in pulmonary edema. Although we did not measure plasma PAF or PAF in the lung tissue, our results indicate that the increase in EVLW after coronary reperfusion was inhibited by PAF-antagonist.

In conclusion, PAF may play an important role in the increase of EVLW after coronary reperfusion.

REFERENCES

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Role of PAF in the Genesesis of Pulmonary Edema


