Korean J Physiol Pharmacol
Vol 4: 515−523, December, 2000

Myocardial Protection by Recombinant Soluble P-selectin Glycoprotein Ligand-1: Suppression of Neutrophil and Platelet Interaction Following Ischemia and Reperfusion

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Polymorphonuclear leukocytes (PMNs) play an important role in myocardial ischemia/reperfusion (MI/R) injury. Moreover, platelets are also important blood cells that can aggravate myocardial ischemic injury. This study was designed to test the effects of PMNs and platelets separately and together in provoking cardiac dysfunction in isolated perfused rat hearts following ischemia and reperfusion. Additional control rat hearts were perfused with 75×10^6 PMNs, with 75×10^6 platelets, or with 75×10^6 PMNs+75×10^6 platelets over a five minute perfusion followed by a 75 min observation period. No significant reduction in coronary flow (CF), left ventricular developed pressure (LVDP), or the first derivative of LVDP (dP/dt max) was observed at the end of the observation period in any non-ischemic group. Similarly, global ischemia (I) for 20 min followed by 45 minutes of reperfusion (R) produced no sustained effects on the final recovery of any of these parameters in any group of hearts perfused in the absence of blood cells. However, I/R hearts perfused with either PMNs or platelets alone exhibited decreases in these variables of 5 ~ 10% ($p<0.05$ from control). Furthermore, I/R hearts perfused with both PMNs and platelets exhibited decreases of 50 to 60% in all measurements of cardiac function ($p<0.01$). These dual cell perfused I/R hearts also exhibited marked increases in cardiac myeloperoxidase (MPO) activity indicating a significant PMN infiltration, and enhanced P-selectin expression on the coronary microvascular endothelium. All cardiaodynamic effects as well as PMN accumulation and P-selectin expression were markedly attenuated by a recombinant soluble PSGL-1 which inhibits selectin mediated cell adhesion. These results provide evidence that platelets and PMNs act synergistically in provoking post-reperfusion cardiac dysfunction, and that this may be largely due to cell to cell interactions mediated by P-selectin. These results also demonstrate that a recombinant soluble PSGL-1 reduces myocardial reperfusion injury by platelet and PMNs interaction.

Key Words: P-selectin, Neutrophils, Platelets, Cardiac dysfunction, Ischemia and reperfusion

INTRODUCTION

Early coronary artery reperfusion plays a key role in myocardial tissue injury associated with acute myocardial infarction. Nevertheless, reperfusion itself can result in enhanced myocardial injury (Tsao et al, 1990; Forman et al, 1993). This reperfusion injury is characterized by a typical inflammatory reaction in which activated polymorphonuclear leukocytes (PMNs) are believed to play an important role (Engler et al, 1986; Tsao et al, 1990). Upon reperfusion, many activated PMNs adhere to the vascular endothelium, and some of them extravasate and accumulate in organs sujected to ischemia and reperfusion (Lefer et al, 1991; Entman & Smith, 1994). Subsequently, activated PMNs induce tissue injury by releasing cytotoxic metabolites including oxygen-derived free

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radicals, eicosanoids, cytokines, and proteolytic enzymes (Weiss, 1989). These concepts are supported by convincing evidence that either rendering animals neutropenic or inhibiting PMN adherence to the vascular endothelium by monoclonal antibodies against cell adhesion molecules can afford significant cardioprotection against myocardial ischemia/reperfusion (MI/R) injury (Romson et al, 1983; Ma et al, 1992; Weyrich et al, 1993).

Exogenous administration of physiological levels of a nitric oxide donor (Pabla et al, 1996) has been found to markedly attenuate the cardiac dysfunction induced by PMNs in perfused rat hearts subjected to ischemia and reperfusion. In these isolated perfused rat heart studies, PMNs were added to plasma and this mixture was added to the Krebs-Henseleit perfusion fluid (Lefer et al, 1997). However, there were no platelets in the cardiac perfusate in these experiments. Platelets are a known source of variety of inflammatory mediators including thromboxane A2 (Hamber et al, 1975), P-selectin (McEver, 1991), and platelet factor-4 (Aziz et al, 1997), all of which upregulate either the complement system or a variety of cytokines (Aziz et al, 1997; Neumann et al, 1997). PMNs also release a variety of cell activating and cytotoxic mediators including cytokines (e.g., TNF α, IL-1 β), complement products (e.g., C5a), proteolytic enzymes (e.g., elastase, cathepsin G) and oxygen derived free radicals (e.g., superoxide) which may contribute to platelet activation (Ferrer-Lopez et al, 1990; Suzuki et al, 1991). However, little is known regarding cross-talk between PMNs and platelets in contributing to the pathophysiology of myocardial ischemia and related cardiovascular disease states.

Recently, Neumann et al (Neuman et al, 1997) have shown that leukocyte-platelet binding is increased in patients with acute myocardial infarction, as well as in patients with unstable angina (Ott et al, 1996). More recently, others have shown that platelets and PMNs interact in regarding vascular tone in arteries injured by angioplasty (Mehri et al, 1997). Nevertheless, the nature of the interaction between platelets and PMNs is not known with regard to their influence on cardiac performance in myocardial ischemia/reperfusion injury or how these two blood cell types communicate with the vasculature.

The major purpose of this study was to investigate the effect of PMNs and platelets, separately and together on cardiac performance in a well controlled established model of ischemia/reperfusion in the isolated perfused rat heart, and to assess the role of recombinant soluble P-selectin glycoprotein ligand 1 (rsPSGL-1) in modulating cardiac dysfunction in blood cell perfused rat heart subjected to myocardial ischemia/reperfusion.

METHODS

Isolated rat heart experiments

Male Sprague-Dawley rats (250~300 g) were anesthetized with 40 mg/kg sodium pentobarbital and administered 1,000 U sodium heparin i.p. (Abbott Laboratories Diagnostic Division). Following a midline thoracotomy, the hearts were rapidly excised, the ascending aorta was cannulated, and retrograde perfusion of the heart was initiated on a Langendorff apparatus at a constant pressure of 80 mmHg. These isolated hearts were perfused with a Krebs bicarbonate buffer of the following composition (in mmol/liter): glucose, 17; NaCl, 120; $NaHCO_3$, 25; $CaCl_2$, 2.5; EDTA, 5.9; and $MgCl_2$, 1.2 maintained at 37°C. The perfusate was oxygenated with 95% O_2+5% CO_2 which equilibrated at a pH of 7.3 to 7.4. Two side arms in the perfusion line located just proximal to the heart inflow cannula allowed infusion of PMNs, platelets and plasma directly into the coronary inflow line. To asses cardiac contractile function, a 2.5 Fr microtip catheter transducer (Millar Instruments, Inc.) was inserted directly into the left ventricular cavity as previously reported (Pabla et al, 1996; Lefer et al, 1997). Left ventricular pressures, maximal rate of development of left ventricular pressure (+dP/dt min), coronary flow, and heart rate were all recorded using a MacLab data acquisition system (ADI Diagnostics Inc.) in conjunction with a Power Macintosh 7600 computer (Apple Computers). All data was stored and analyzed at the end of each experiment.

Rat neutrophil isolation

Neutrophil donor rats (300~350 g) received a 10 ml injection of 0.5% glycogen i.p. (Sigma Chemical Co.). Eighteen hours later, the rats were anesthetized with ethyl ether and the neutrophils were harvested by peritoneal lavage in PBS. The peritoneal lavage was centrifuged at 3,000 rpm and 4°C for 10 minutes as previously described (Lefer et al, 1997). Finally,

the PMNs were washed in Krebs buffer and counted using a microscope and hemocytometer. These neutrophil preparations were >95% pure, and >95% viable using exclusion of 0.3% trypan blue as the criterion for viability. Furthermore, PMNs obtained by this method have been found to respond normally in cell adhesion tests (Lefer et al, 1997).

Additional neutrophils were isolated from blood according to the method of Williams et al (Williams et al, 1987) using the hetastarch exchange transfusion technique in 400 g rats anesthetized with pentobarbital sodium 40 mg/kg administered i.p.. Yields of 120×10^6 PMNs per rat were obtained which were > 95% pure and 95% viable using the trypan blue exclusion test. These blood PMNs were washed 5-6 times with PBS to remove the hetastarch prior to use.

Rat platelets and plasma

Whole blood was obtained by performing an intracardiac puncture in anesthetized rats with a 20 ml plastic syringe with a 20 gauge needle (Becton Dickinson Co.) containing 2,000 U sodium heparin. To obtain platelets, the whole blood was immediately spun in a refrigerated centrifuge (GSGR; Beckman Instruments, Inc.) at 900 rpm for the plasma was decanted from the platelets. Next, the platelets were resuspended in Krebs buffer and counted. Platelet preparations were >95% pure.

Perfused heart experimental protocol

After a 15 minute stabilization period, baseline left ventricular developed pressure (LVDP), +dP/dt max., and coronary flow were measured every 5 minutes for 15 minutes to ensure complete equilibration of the hearts. Flow of Kreb's buffer was then reduced to zero, creating global, total ischemia. This ischemia was maintained for 20 minutes. Reperfusion of the hearts was instituted by restoration of flow of buffer to the heart to that of pre-ischemic levels. At 5 minutes of reperfusion, either 75×10^6 PMNs alone, 75×10^6 platelets alone, or both cell types together were infused into the hearts. The blood cells were infused over a 5 minute period directly into the coronary circulation via a set of side ports situated in the perfusion line just proximal to the inflow cannular. The PMNs were suspended in 5 ml of Krebs buffer in 5 ml syringe and the platelets were suspended in 5 ml of plasma and also placed in a separate 5 ml syringe located just distal to the platelet inflow port and just proximal to the inflow port to the coronary circulation. The hearts were allowed to reperfuse for a total of 45 minutes during which time data were collected every 5 minutes for the first 30 minutes and a the 45 minute time point. We used a recombinant soluble P-selectin ligand (rsPSGL-1) decribed by Takada et al. (Takada et al, 1997) which acts as a specific P-selectin inhibitor. A rsPSGL-1, 5 μg/ml was infused over the first 5 minutes of reperfusion. Finally, we perfused five rat hearts with 75 $\times 10^6$ PMNs alone, 75×10^6 platelets alone, or both cell types together isolated from blood as described above.

Determination of cardiac tissue myeloperoxidase

Myocardial tissue myeloperoxidase (MPO), an enzyme occurring virtually exclusively in neutrophils, was determined as described previously (Mullane et al, 1985). One unit of MPO is defined as the quantity of enzyme hydrolyzing 1 mmol of peroxide per minute at 25°C. The assays were performed without knowledge of the group from which each sample originated.

Histology and immunohistochemistry

In addition to the hearts used for assessment of left ventricular function, additional rat hearts were perfused to determine the number of PMNs infiltrating into the heart by histological methods. After 45 minutes of reperfusion, hearts were removed from the perfusion apparatus and placed in 4% paraformaldehyde overnight at 4°C. The heart was cut into sections and dehydrated using graded acetone washes at 4°C. Tissue sections were embedded in plastic (Immunobed; Polysciences Inc.), and 4 μm thick sections were cut and transferred to Vectabond-coated slides (Vector Laboratories, Inc.). The slides were soaked in 95% ethanol for 10 minutes to remove some of the plastic embedding and to allow staining of the tissue. After the 10 minute ethanol wash, the tissue sections were stained with either hematoxylin solution Gill No. 3 for 10 minutes (Sigma Chemical Co.) or Giemsa stain for 3 minutes (Sigma Chemical Co.). The slides were then observed microscopically and the numbers of PMNs and platelets were counted and tallied. Five fields from each of two slides were counted from each heart, and three rats were studied in each group.

Immunohistochemistry for P-selectin was performed on tissues according to previously described techniques in 3 to 4 hearts from each group (Lefer et al, 1997). The basic method employed was the avidin-biotin immunoperoxidase technique employing mAb PB 1.3 as the monoclonal antibody directed against P-selectin. Positive staining was defined as a coronary microvessel displaying brown reaction on >50% of the circumference of its endothelium. Fifty vessels/tissue sample were examined in each of 3 to 4 hearts per group.

Statistical analysis

All data are presented as mean±SEM. Data were compared by ANOVA using post-hoc analysis with fisher's corrected test. The data on coronary flow and left ventricular function were analyzed by ANOVA incorporating repeated measures. A probability value of 0.05 was considered statistically significant.

RESULTS

Rat hearts were perfused at control flow for 80 minutes, or for 15 minutes of control flow followed by 20 minutes of total global ischemia and 45 minutes of reperfusion at near control flows either with or without blood cell, (PMNs and platelet) and plasma. Perfusion of rat hearts was performed at a perfusion pressure of 80 mmHg. In the absence of either PMNs or platelets resulted in a completely normal coronary flow (CF), left ventricular developed pressure (LVDP) and first derivative of LVDP (dP/dt max) at the end of the 80 minutes observation period. Moreover, perfusion of control nonischemic hearts without blood cells, with PMNs alone, with platelets alone, or with both PMNs and platelets did not alter any index of cardiac function measured. Thus, this perfused rat heart model subject to ischemia/reperfusion does not result acutely in either a flow deficit or a cardiac dysfunction in the absence of blood cells. Conversely, perfusion of hearts with blood cells in the absence of ischemia/reperfusion also exerted no coronary flow deficit or cardiac dysfunction. However, ischemic hearts reperfused in the presence of PMNs or platelets exhibited slight but significant adverse cardiodynamic effects (i.e., reduced coronary flow, cardiac dysfunction). Reperfusion of ischemic hearts with both PMNs (75×10^6) and platelets (75×10^6) produced profound deficits in coronary flow and a dramatic degree of cardiac dysfunction. When elicited rat PMNs isolated by peritoneal lavage were compared with rat PMNs isolated from blood, the blood PMNs (100×10^6) and peritoneal PMNs (100×10^6) showed almost the same cardiac dysfunction in LVDP as exhibited by the 50±3% and 52±4% recovery of initial value (data not shown).

Ischemic/reperfused hearts perfused with PMNs or platelets alone exhibited significant effects. Coronary flow returned to 89±3% of control in the presence of 75×10^6 PMNs and to 93±2% of control in the

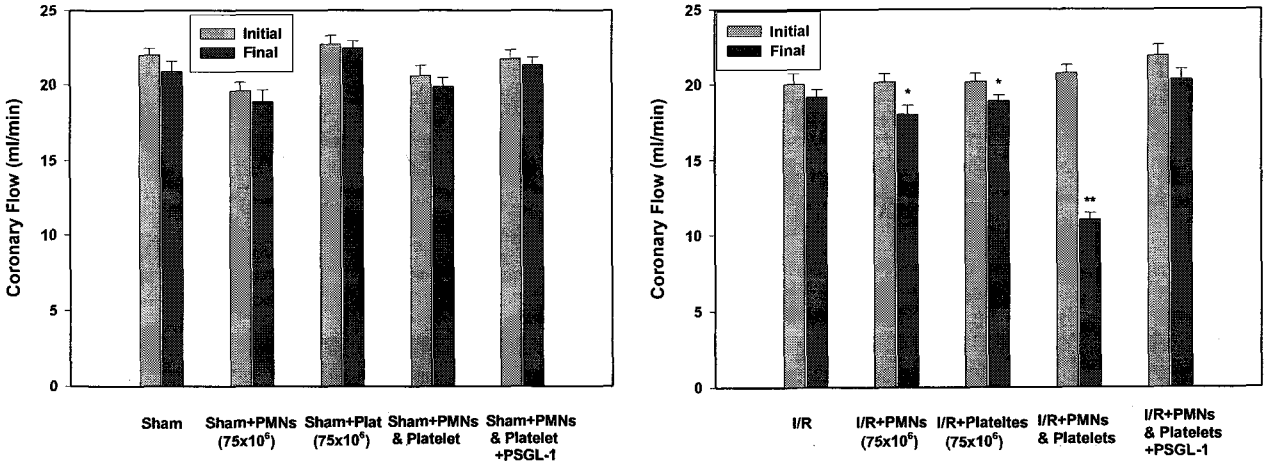

Fig. 1. Initial and final coronary flow (CF) expressed in ml/minute in isolated perfused rat heart before ischemia and after reperfusion. Ischemic hearts were perfused in the presence or absence of PMNs and/or platelets. All values are expressed as means±SEM, n=5. *$p<0.05$, **$p<0.01$

presence of 75×10^6 platelets ($p<0.05$ in both cases from initial values) (Fig. 1). However, when the same numbers of PMNs and platelets were co-perfused in I/R hearts, coronary flow returned to only $53 \pm 4\%$ of control, a value significantly lower than in any other group ($p<0.01$ from initial). This effect represents a synergistic relationship between platelets and neutrophils, since their effects are two times greater than the additive effects of platelets and PMNs give alone (Fig. 1). Nevertheless, perfusion under these conditions with rsPSGL-1 at 5 μg/ml resulted in a decrease in coronary flow of only $7 \pm 3\%$ of initial value.

Similar results were obtained with regard to left ventricular cardiodynamics (i.e., LVDP, Fig. 2). $10 \pm 3\%$ in hearts perfused with PMNs and $5 \pm 2\%$ in hearts perfused with platelets ($p<0.05$, from initial) (Fig. 2). However, perfusion with both PMNs and platelets resulted in a decline in left ventricular developed pressure by $52 \pm 3\%$ ($p<0.01$ from initial and from all other groups) again indicating a marked degree of synergy between PMNs and platelets. Moreover, addition of rsPSGL-1 to the perfusate containing

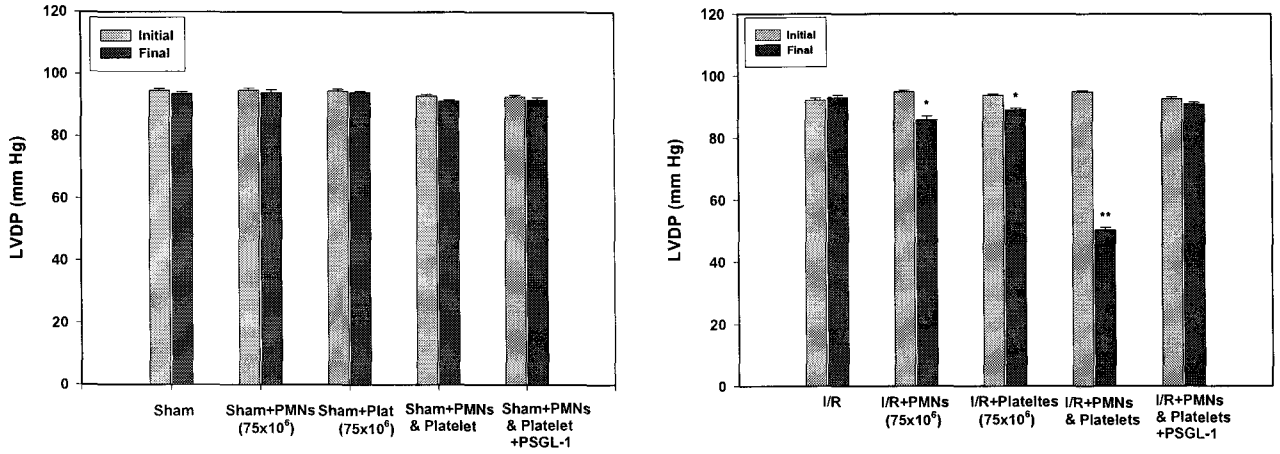

Fig. 2. Initial and final left ventricular developed pressure (LVDP) expressed in mmHg in isolated perfused rat hearts suject to 20 minutes of global ischemia and reperfusion. Ischemic hearts were reperfused in the presence (PMNs: 75 million; and platelets; 75 million) or absence of PMNs and/or platelets. PMNs markedly decreased the recovery of the hearts throughout reperfusion, which was worsened by the platelets. All values are expressed as means $\pm$ SEM, n=5. *$p<0.05$, **$p<0.01$

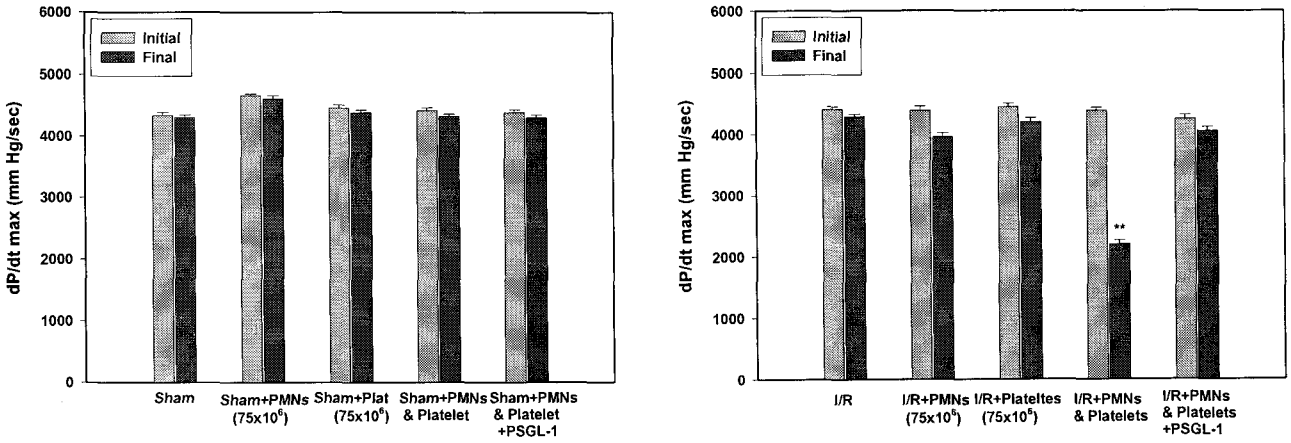

Fig. 3. Initial and final values for the first derivative of LVDP (+dP/dt max) expressed in mmHg/sec. in rat hearts subject to ischemia and reperfusion. Ischemic hearts were perfused in the presence or absence of PMNs and/or platelets. PMNs significantly impaired the heart function as seen in the depression of +dP/dt max, which was aided by the platelets. All values are expressed as means $\pm$ SEM, n=5. **$p<0.01$

both PMNs and platelets completely blocked this cardiac dysfunction.

As would be expected, the first derivative of LVDP (i.e., dP/dt max) exhibited the same relationship as did the basic LVDP data (Fig. 3). Thus, coronary perfusion with PMNs or with platelets resulted in decreases in dP/dt max of $9\pm4\%$ and $5\pm3\%$ (not significantly different from initial), whereas the combination of platelets and PMNs resulted in a reduction in dP/dt max by $50\pm4\%$ ($p<0.01$ from initial or any other group) reinforcing the dramatic degree of synergy between PMNs and platelets in inducing cardiac dysfunction. Perfusion with the anti-selectin agent (i.e., rs PSGL-1) in combination with platelet and PMNs attenuated the decrease in dP/dt max to $5\pm3\%$ ($p<0.01$ from I/R+PMNs+platelets) (Fig. 3).

We also collected the hearts at the end of the reperfusion period, froze them at $-70^{\circ}C$ and analyzed cardiac tissue for myeloperoxidase (MPO) activity as an index of accumulated PMNs. No MPO activity could be detected in control hearts or any ischemic/reperfused heart not perfused with PMNs, thus indicating that there are very few resident PMNs in the perfused rat hearts. However, Fig. 4 shows that perfusion of ischemic reperfused hearts with 75×10^6 PMNs increased MPO activity significantly ($p<0.01$ from ischemia/reperfusion alone). Furthermore, coperfusion of I/R hearts with PMNs and platelets increased the MPO activity 2.3 fold ($p<0.01$ from I/R +PMNs). However, addition of rsPSGL-1 to the PMNs and platelets perfusing I/R hearts dramatically attenuated MPO activity to values below those observed for I/R+PMNs alone (Fig. 4).

As an additional verification of the MPO activity, we performed histological analysis of perfused rat hearts, and counted PMNs and platelets in these sections. Essentially no resident leukocytes were observed in perfused rat hearts subjected to ischemia reperfusion under the conditions of the current protocol. However, when the hearts were perfused with 75×10^6 PMNs, over 50 PMNs could be detected per field, on each section. The percentage of intravascular PMNs was 83%. No extravascular platelets were observed. Perfusion with 75×10^6 PMNs and 75×10^6 platelets resulted in significantly greater numbers of both PMNs and platelets on each microscopic field (data not shown). Similar results were obtained with MPO activity.

Fig. 5 illustrates the degree of expression of P-selectin on coronary venules in perfused rat hearts. No P-selectin expression was observed in any non-ischemic perfused rat hearts. Moreover, very low ex-

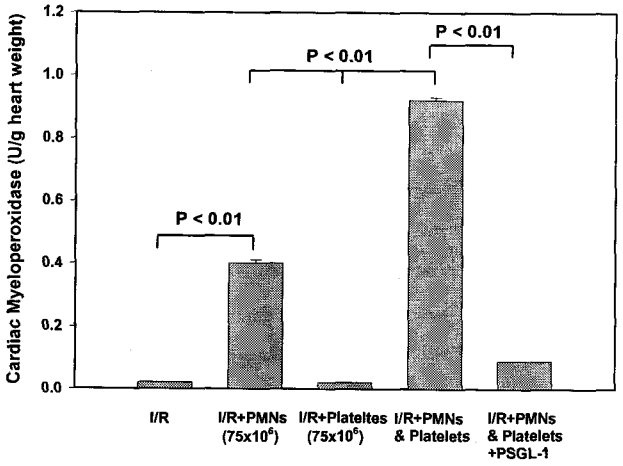

Fig. 4. Cardiac myeloperoxidase (MPO) activity in cardiac tissue samples obtained from sham-operated non-ischemic, and from ischemic-reperfused rat hearts, with PMNs and/or platelets. MPO activity is expressed in units per gram of wet tissue weight. All values are means±SEM. rsPSGL-1 significantly inhibited the increased MPO activity in hearts perfused with PMNs and platelets.

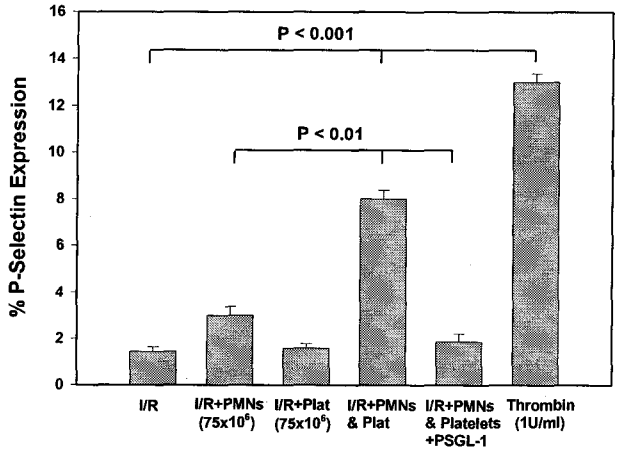

Fig. 5. P-selectin expression in coronary vascular endothelium of isolated perfused rat hearts. All values are means±SEM of fifty sections each in three to four hearts/group. A positive vessel is one in which >50% of its endothelial surface exhibits a peroxidase brown reaction product. Perfusion with platelets+PMNs significantly upregulated coronary endothelial P-selectin expression in ischemic reperfused hearts ($p<0.01$). This was significantly attenuated by rsPSGL-1 ($p<0.01$).

pression was observed in ischemic-perfused rat hearts perfused without blood cells (i.e., 1.4% of the vessels). The presence of 75×10^6 PMNs or 75×10^6 platelets increased P-selectin expression modestly, but perfusion with both PMNs and platelets resulted in a marked increase in P-selectin expression on the coronary vascular endothelium ($p < 0.01$). This enhanced expression of P-selectin was markedly attenuated by co-perfusion with rsPSGL-1 ($p < 0.001$). Therefore it was concluded that P-selectin up-regulation on the ischemic-reperfused coronary vascular endothelium occurs in the presence of platelets and neutrophils, and that this is attenuated by rsPSGL-1, which inhibits selectin-mediated interaction between platelets and PMNs, platelets and the endothelium, and PMNs and the endothelium.

DISCUSSION

The results obtained in this study clearly point toward two important conclusions. First, circulating neutrophils at the time of reperfusion significantly contribute to post-reperfusion cardiac contractile dysfunction. Secondly, blood platelets act in a cooperative manner synergizing with PMNs to exacerbate this cardiac contractile dysfunction. Recently, other reports suggesting PMNs-platelet interaction of patient with coronary artery disease have appeared (Ott et al, 1996; Neumann et al, 1997). In this regard, leukocyte-platelet binding has recently been shown to occur in patient either with unstable angina (Ott et al, 1996) or following acute myocardial infarction (Neumann et al, 1997). The basis for this cell to cell interaction is not known, but may involve P-selectin up-regulation on platelet or endothelial cell membranes (Rinder & Fitch, 1996). This concept is consistent with the finding that thrombin stimulation of platelets leads to increase expression of pro-inflammatory cytokines (e.g., interleukin-1 and interleukin-8) by leukocytes (Neumann et al, 1997). Thus P-selectin may mediate platelet-neutrophil interaction following ischemia-reperfusion, such as that observed in the perfused rat hearts employed in the present study. Additional possibilities are that leukocytes and platelets interact and cooperate metabolically to facilitate transcellular biosynthesis of some mediator of inflammation, as is known to occur in the case of the leukotrienes (Lindgren & Edenius, 1993).

Our results add to our knowledge of cell to cell interaction in the pathophysiology of reperfusion injury. First, the interaction between PMNs and platelets is a synergistic one in which the response of the two cell types together is greater than that which would be predicted by the same numbers of the two cell types perfused separately. This synergism is clearly suggestive of a humoral interaction amplifying the actions of the two cell types, an effect which could be mediated, at least in part, by the selectin family of adhesion glycoproteins. Secondly, blockade of the selectin family can markedly attenuate this synergism between platelets and neutrophils. This was demonstrable using a rsPSGL-1, blocking one of the major high affinity ligand for P-selectin (Moore et al, 1992). rsPSGL-1 has been shown to be effective in attenuating renal tissue injury in a rat model of renal ischemia-reperfusion (Takada et al, 1997). This anti-selectin agents could exert protective effects in one of several ways. Initially, this agents could have inhibited platelet P-selectin mediated effects between platelets and neutrophils, or between platelets and endothelial cells. Alternatively, they could have inhibited endothelial P-selectin mediated effects between endothelial cells and neutrophils. Probably, the overall protective effect of the anti-selectin agents is a combination of all of these possibilities since all three cell types are intimately involved in the pathophysiology of ischemia/reperfusion (Lefer & Lefer, 1993). Clearly, the adherence of PMNs to the endothelium is central to much of the ensuing pathophysiology (Ma et al, 1992; Weyrich et al, 1993), whether it occurs as single PMNs or clusters of platelet-neutrophil complexes. The third major conclusion derived from these studies is that blood cells and mediators on their surface or released by these cells can induce significant effects on myocardial contractility in sensitized hearts (i.e., ischemia/reperfusion). Thus, in our model, myocardial ischemia/reperfusion in the absence of blood cells produced no deficit in cardiac contractility. When PMNs alone or platelets alone were added to the system, modest deficits in contractility occurred. PMNs isolated from the blood were comparable to elicited PMNs indicating that partially activated PMNs contain sufficient mediators to produce significant cardiac dysfunction. Only when both cell types were given together at reperfusion, was there a marked decline in coronary flow and cardiac contractility.

One may speculate as to the cellular mechanism of this PMN-platelet co-operativeness in producing post-

reperfusion cardiac dysfunction. Several possibilities exist. First, PMN or platelet aggregates or PMN-platelet complexes could obstruct flow in significant numbers of coronary microvessels and plug up these vessels, thus interfering with the normal distribution of coronary flow to the cardiac myocytes. Regarding this possibility, we saw no evidence of obstruction in any of the histological sections we observed. However, we did observe small numbers of homotypic (i.e., platelet-platelet or PMN-PMN) or heterotypic (i.e., PMN-platelet) aggregates, but these were scattered and were not large enough to physically obstruct coronary vessels. There is evidence of this plugging up of vessels in vivo (Engler et al, 1983), but under the conditions of our experiments, this did not occur. With regard to pro-inflammatory humoral mediators, both platelets and PMNs are rich in potential humoral candidates. Platelets have abundant pro-thrombotic and vasoconstrictor agents including thrombin, histamine, thromboxane A2 and platelet factor-4. Several of these humoral agents could contribute to the reduced coronary flow observed in the present experiments that could lead to cardiac contractile dysfunction. Many of these agents also up-regulate P-selectin expression by translocation of P-selectin protein from α-granules to the surface of the platelet membrane (Lorant et al, 1991; Lefer et al, 1994). Moreover, neutrophils release a variety of cytokines, proteases, lipid mediators, and oxygen-derived free radicals (Lorant et al, 1991; Lefer et al, 1994) which can interact with platelet derived mediators. This combination of mediators could exacerbate both the reduced coronary flow and the cardiac contractile dysfunction observed in the present experiments.

P-selectin expressed on platelet membranes could bind neutrophils to platelets and both cell types to the endothelium which also expresses P-selectin on its surface translocated there from Weibel-plade bodies (Lorant et al, 1991). We observed a significant increase in P-selectin expression on ischemic-reperfused coronary vascular endothelium in the presence of PMNs and platelets, and this was markedly attenuated by rsPSGL-1. Under the conditions of the present flow-through experiment, we could not accurately measure release of humoral agents could up-regulate P-selectin. However, our results are consistent with the presence of humoral mediators. In any case, we have clear data to support the third hypothesis, that platelets and PMNs synergistically promote enhanced endothelial adhesion and eventual transendothelial migration of PMNs into the ischemic reperfused myocardium. We observed both significantly elevated cardiac MPO activity, a specific marker of neutrophils, and increased numbers of PMNs were observed microscopically in ischemic hearts reperfused with both cell types compared to those reperfused with either cell type alone. The net result is to augment and exacerbate the effects of neutrophils on propagating cardiomyocyte injury following reperfusion of the ischemic myocardium. One factor contributing to this phenomenon could be the ability of activated PMNs to stimulate platelet function (DeGaetano et al, 1990). Therefore, these results suggest that either anti-platelet or anti-neutrophil agents may provide some benefit against reperfusion injury. Moreover, dual anti-platelet and anti-neutrophil therapy or an agent that addresses both cell types may be of particular utility in myocardial ischemia-reperfusion.

ACKNOWLEDGEMENT

This study was supported by Chung-Ang University Research Grants in 2000. We gratefully acknowledge the gift of rsPSGL.Ig from Gray Shaw of Genetics Institute, Cambridge, MA.

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