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LEUKOCYTES, OXYGEN RADICALS, AND MYOCARDIAL INJURY DUE TO ISCHEMIA AND REPERFUSION*

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Abstract—Ischemic myocardium generates stimuli for neutrophil chemotaxis before the final extent of irreversible ischemic injury is attained. Reperfusion accelerates the infiltration of ischemic myocardium by neutrophils. Oxygen radicals released by the activated neutrophils may exacerbate the tissue damage caused by ischemia. Neutrophil depletion by antiserum was shown to limit infarct size in dogs undergoing coronary occlusion for 90 minutes followed by reperfusion for 6 or 72 hours, but not in dogs undergoing occlusion for 4 hours. Prostacyclin, which inhibits the generation of superoxide anions by neutrophils, also limited canine myocardial injury despite no effect on collateral blood flow. Iloprost, an analogue of prostacyclin that inhibits neutrophils also reduced infarct size, while SC39902, an analogue that does not inhibit neutrophils, did not alter infarct size. The results suggest that oxygen radicals released by activated neutrophils play a role in the pathophysiology of myocardial injury due to ischemia followed by reperfusion.

Keywords—Myocardial ischemia, Myocardial infarction, Reperfusion injury, Leukocytes, Neutrophils, Oxygen free radicals, Prostacyclin, Iloprost

Recent multicenter clinical trials demonstrated that hospital mortality is reduced by the early administration of streptokinase to patients with acute myocardial infarction.[1,2] Thus, thrombolytic therapy will likely be recognized as the standard of care for patients who are suspected of having coronary artery thrombosis. There is increasing experimental evidence, however, that reperfusion is a "double-edged sword."[3] While it is true that myocardial cells will inevitably die without adequate oxygen and nutrients, reperfusion may also accelerate pathophysiologic processes that contribute to irreversible myocardial damage. The potential role of leukocytes and oxygen radicals in the pathophysiology of myocardial injury due to regional ischemia and reperfusion is an area of intense investigation. The purpose of this essay is to critically review recent data that either support or oppose the hypothesis that oxygen radicals released by activated leukocytes exacerbate tissue damage precipitated by coronary artery occlusion.

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MEDIATORS OF LEUKOCYTE-INDUCED INJURY

Leukocytes can release a variety of mediators capable of promoting tissue injury, including proteolytic enzymes, platelet-activating factor (PAF), arachidonic acid metabolites, and activated species of oxygen (Table 1). The influence of leukocyte-mediated proteolysis on the extent of myocardial necrosis is uncertain. The infarcted myocardium of leukopenic rats showed significantly less degradation of collagen, a component of the heart's fibroskeleton.[4] Although collagen degradation was attributed to inflammatory cell proteases,[4] oxygen free radicals can also degrade collagen.[5] Also, Bolli and co-workers [6,7] found that the suppression of proteolysis in ischemic rat myocardium did not alter infarct size, indicating that proteases may not play a primary role in the extent of myocardial infarction.

PAF released by leukocytes or present in endothelial cells may alter tissue perfusion by stimulating platelets to aggregate and release vasoactive substances.[8,9] PAF also triggers neutrophil aggregation, degranulation, and chemotaxis, and augments the generation of oxygen radicals in response to some stimuli.[10] The release of PAF may also result in the depression of myocardial contractility.[11,12] The effect of PAF antagonists during experimental myocardial ischemia has not been reported.

Activated neutrophils and macrophages release leukotrienes, products of the lipoxygenase pathway of arachidonic acid metabolism. Leukotrienes C, D, and E, the predominant leukotrienes synthesized by macrophages, have been shown to cause coronary vasoconstriction.[13–16] Leukotriene B4, the major leukotriene formed by neutrophils, lacks direct effects on the coronary circulation and myocardial contraction,[13] but promotes tissue damage by stimulating the chemotaxis of neutrophils[17] and their adherence to vascular endothelium.[18] Inhibitors of the lipoxygenase pathway, such as BW 755C[19,20] and nafazatrom,[21,22] were shown to limit canine infarct size. Lipoxygenase inhibitors may directly inhibit the activation of leukocytes and their release of oxygen radicals, however, making it impossible to conclusively relate their salutory effects to the inhibition of leukotriene synthesis.[22] Selective inhibitors of leukotriene synthesis are not yet available.

The role of oxygen radicals in neutrophil-mediated tissue injury is well documented and has been reviewed extensively.[23,24] Oxygen radicals can cause tissue injury in multiple ways, including the peroxidation of membrane lipids, the denaturation of proteins, and the impairment of ATP synthesis. There is increasing evidence that treatment with oxygen radical scavengers limits experimental myocardial injury due to regional ischemia and reperfusion.[25] Thus, activated species of oxygen are potential mediators of leukocyte-induced myocardial injury.

MYOCARDIAL EFFECTS OF ACTIVATED LEUKOCYTES

Several studies have examined the effects of leukocyte-derived oxygen radicals on myocardial function. Rowe and co-workers[26] incubated sarcoplasmic reticulum isolated from canine myocardium with human leukocytes activated by phorbol myristate acetate (PMA). The activated leukocytes inhibited calcium transport and the ATPase activity of the sarcoplasmic reticulum. Free radical scavengers prevented the inhibitory effects of the leukocytes, indicating that neutrophil-derived oxygen radicals interfered with the function of the sarcoplasmic reticulum. Gillespie and colleagues[27] studied the effects of neutrophils on Langendorff-perfused rabbit hearts. The addition of PMA-stimulated neutrophils to the perfusate evoked increases in coronary resistance and decreases in left ventricular pulse pressure that were attenuated by scavengers of oxygen radicals. Endothelial cell injury and death caused by activated neutrophils may also involve oxygen free radicals.[28] Although there are no published studies of the effects of neutrophils on cultured myocytes, Scott et al.[29] showed that hyperoxia-induced injury to cultured myocytes may be due to oxygen free radicals.

Table 1. Potential Mediators of Leukocyte-Induced Myocardial Injury

Mediator	Effect
Proteases	digestion of proteins ? activation of complement
Platelet-activating factor	platelet activation neutrophil activation
Leukotrienes	neutrophil chemotaxis, adherence coronary vasoconstriction
Oxygen metabolites	peroxidation of membrane lipids denaturation of proteins impairment of ATP synthesis

ACCUMULATION OF LEUKOCYTES WITHIN ISCHEMIC MYOCARDIUM

Cultured myocytes that are rendered hypoxic release products that are chemotactic for neutrophils.[30] Chemotactic activity for neutrophils is detectable in coronary sinus blood within 30 minutes of occlusion of a canine coronary artery.[31] Rossen et al.[32] used radioactive tracers to demonstrate that the uptake of neutrophils and complement fragments by ischemic canine myocardium begins within 45 min of coronary artery occlusion. Subsequently, they showed that ischemic cardiac muscle releases peptides that react with the first component of complement.[33] Ischemic myocardial necrosis after occlusion of a canine coronary artery begins within 40 min after occlusion, and in the absence of reperfusion, the full extent of irreversible ischemic injury is not attained for at least 3 to 6 h.[34] Thus, ischemic myocardium generates stimuli for neutrophil chemotaxis long before the entire ischemic region undergoes irreversible injury.

Reperfusion accelerates the infiltration of ischemic myocardium by neutrophils.[35] The ultimate extent of necrosis after temporary coronary occlusion followed by early reperfusion may not be reached until several hours after reperfusion. Therefore, the data suggest that the release of chemotactic factors and the infiltration of neutrophils occur earlier enough to participate in the destruction of cells that escape lethal ischemic injury due to the deprivation of oxygenated blood, the

accumulation of toxic products of cellular metabolism, and associated abnormalities of cellular electrolyte composition and volume regulation.[34]

EFFECTS OF NEUTROPHIL DEPLETION DURING MYOCARDIAL ISCHEMIA

Multiple studies have examined the effects of neutrophil depletion during myocardial ischemia and reperfusion. Romson et al.[36] demonstrated a 40% reduction of canine infarct size associated with antiserum-induced neutropenia maintained throughout 90 min of ischemia followed by 6 h of reperfusion. Mitsos et al.[37] and Jolly et al.[38] also measured infarct size in dogs administered anti-neutrophil antiserum. After coronary artery occlusion for 90 minutes followed by reperfusion for 6 hours, the extent of infarction expressed as a percentage of the area at risk was 46% in the control group and 31% in the neutropenic group.[37] Similar results were obtained in dogs subjected to 90 min of ischemia followed by 24 h of reperfusion despite a 60% recovery of the circulating neutrophil count between 10 h (the last antiserum injection) and 24 h (the time of sacrifice).[38] Mullane et al.[20] reported a significant reduction of infarct size in dogs made neutropenic by the administration of hydroxyurea before a 1 h coronary occlusion followed by 4 h of reperfusion. Recently, Simpson and Lucchesi (unpublished observations) observed a sustained limitation of infarct size by neutrophil depletion in dogs undergoing a 90 min period of ischemia followed by 72 h of reperfusion, suggesting that neutrophil depletion does not merely delay the necrosis of irreversibly-injured tissue.

There are conflicting results from studies of neutrophil depletion during coronary occlusions longer 90 min. Jolly et al.[38] found no difference between control and antiserum-treated dogs subjected to 4 h of ischemia followed by reperfusion. Preliminary data reported by Ksiezycka et al.[39] showed that neutrophil depletion limited canine myocardial injury measured after coronary occlusion lasting 6 hours.

Similarly, conflicting data exist with respect to the effects of neutrophil depletion on the recovery of myocardial function after coronary occlusion for 15 min. Both Bolli and colleagues[40] and Shea and co-workers[41] reported that neutropenia induced by neutrophil antiserum failed to alter the extent of contractile dysfunction of myocardium subjected to 15 min of ischemia and 4 h of reperfusion. On the other hand, Engler et al.[42] observed full recovery of systolic function when myocardium was reperfused with blood depleted of leukocytes by filtration after 15 min of regional ischemia. Three independent investigations found that treatment with the combination of superoxide dismutase and catalase enhances the recovery of left ventricular function after coronary occlusion for 15 min.[43-45] Thus, neutrophils are probably not the sole source of oxygen radicals within reperfused canine myocardium.

EFFECTS OF NEUTROPHIL INHIBITION

Cyclooxygenase inhibitors and cyclooxygenase metabolites have played an instrumental part in elucidating the role of neutrophils in the pathophysiology of ischemic myocardial injury. Ibuprofen, an inhibitor of cyclooxygenase, limited canine myocardial injury despite no demonstrable effects on myocardial oxygen demand or regional myocardial blood flow.[46] One postulated mechanism of action was an anti-platelet effect. Treatment with aspirin, however, did not reduce canine infarct size.[47] Two recent studies showed that platelet depletion with platelet anti-serum did not limit experimental canine myocardial infarction.[48,49] Therefore, platelet inhibition seems to have no beneficial effect on myocardial injury due to coronary occlusion-reperfusion. Also, ibuprofen did not affect the accumulation of platelets within canine myocardium subjected to ischemia and reperfusion.[50]

An alternative hypothesis was that ibuprofen might suppress the myocardial accumulation of neutrophils. Consequently, Romson et al.[50] injected dogs with indium-111-labeled neutrophils before temporary occlusion of the left circumflex coronary artery. The activity of indium-111 within the area at risk of infarction was significantly less in ibuprofen-treated dogs than in control dogs. Subsequently, Flynn et al.[51] compared the effects of two cyclooxygenase inhibitors, ibuprofen and aspirin, on neutrophil function and myocardial infarction in cats. Ibuprofen inhibited neutrophil function and decreased infarct size, while aspirin was devoid of effects on either neutrophils or myocardial injury. Thus, inhibition of neutrophil function rather than platelet or cyclooxygenase metabolism appears to explain the myocardial protection afforded by ibuprofen.

The cardioprotective property of prostacyclin (PGI_2), a product of the cyclooxygenase pathway of arachidonic acid metabolism, may also be attributed to the inhibition of neutrophils. Early studies concluded that PGI_2 limited canine infarct size by enhancing collateral blood flow to ischemic myocardium.[52] Subsequently, it was shown that PGI_2 also reduced infarct size in the absence of an increase in collateral blood flow during coronary artery occlusion.[53] Recently, Simpson and colleagues[54-56] re-examined the mechanism of prostacyclin's salutory effect on experimental myocardial infarction. Dogs were treated with continuous infusions of PGI_2 or a stable

PGI_2 analogue, SC39902, beginning 30 min before and ending 2 h after a 90 min occlusion of the left circumflex coronary artery.[54] The compounds caused equivalent decreases in blood pressure, but only PGI_2 reduced the extent of myocardial injury (Fig. 1). The analogue also differed from PGI_2 in its effect on neutrophils. PGI_2, but not SC39902, inhibited the generation of superoxide anions by canine neutrophils stimulated with opsonized zymosan (Fig. 1).

Simpson et al.[55] also investigated the effects of iloprost, another prostacyclin analogue, in a canine model of coronary occlusion-reperfusion. Iloprost was similar to PGI_2 in that it significantly limited the extent of myocardial injury after 90 min of ischemia and 6 h of reperfusion, and inhibited the generation of superoxide anions by canine neutrophils in vitro (Fig. 1). The effect of iloprost on neutrophil migration in vivo also was studied. Treatment with iloprost reduced the myeloperoxidase activity, an index of neutrophil accumulation, within the zone of myocardial ischemia and in inflammatory skin lesions produced by the intradermal injection of zymosan-activated plasma, a stimulus for neutrophil chemotaxis.

Further experiments compared the effects of short and long-term treatment with iloprost on canine infarct size measured 72 h after reperfusion.[56] A continuous infusion of iloprost until 48 h after reperfusion was associated with significantly lower blood neutrophil counts and smaller infarcts than the infusion of vehicle. In dogs receiving iloprost for only the first 2 h of reperfusion, the neutrophil counts were similar to those of control dogs within 24 h of reperfusion, and the extent of infarction did not differ from the controls after 72 h of reperfusion. Thus, short-term treatment with iloprost limited myocardial injury measured after 6 h of reperfusion,[55] but not after 72 h of reperfusion,[56] suggesting that neutrophils are capable of increasing the ultimate extent of myocardial injury even later than 6 hours after the reperfusion of ischemic myocardium.

LEUKOCYTES, OXYGEN RADICALS, AND MYOCARDIAL SCAR FORMATION

Drugs with beneficial short-term effects on infarct size may also have deleterious long-term effects on infarct healing. Glucocorticoids, for example, were

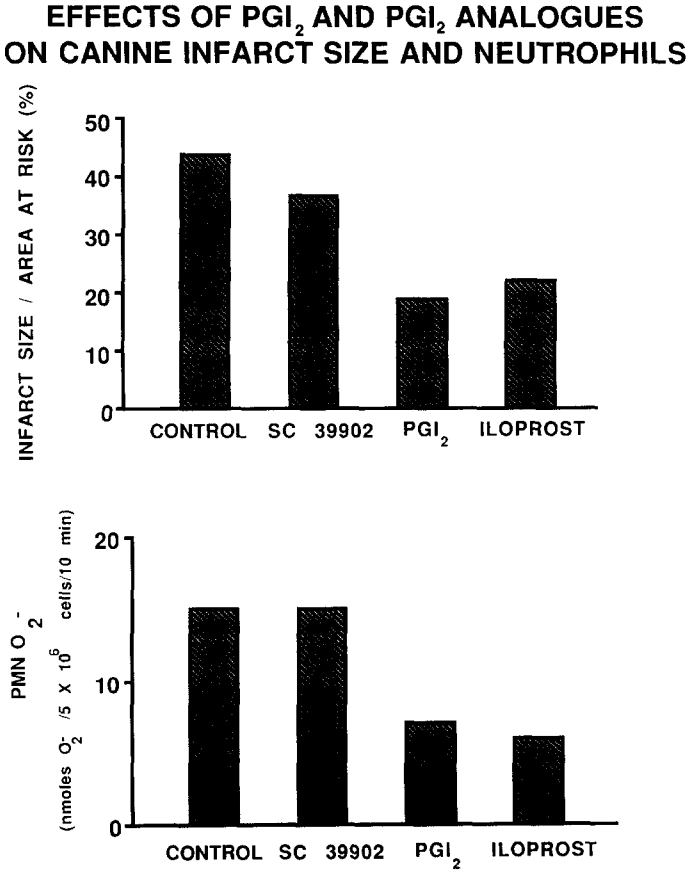

Fig. 1. Effects of prostacyclin (PGI_2) and the PGI_2 analogues iloprost and SC39902 on canine infarct size and neutrophils. Anesthetized dogs underwent coronary artery occlusion for 90 minutes followed by reperfusion for 6 hours. Canine neutrophils were activated with opsonized zymosan. PGI_2 and iloprost significantly reduced infarct size and neutrophil superoxide anion generation (PMN O_2^-) while SC39902 did not.

shown to limit experimental infarct size[57] but also were reported to cause infarct thinning in rats[57] and dogs,[58] and ventricular aneurysm formation in man.[59] Ibuprofen, a non-steroidal anti-inflammatory agent, was found to promote thinning of infarcted canine myocardium.[60,61] Aspirin, in contrast, did not impair myocardial scar formation.[60] Ibuprofen inhibits neutrophil function, while aspirin does not, suggesting that the inhibition of neutrophil functions might underlie the adverse effect on infarct healing.[51] On the other hand, neither superoxide dismutase, a scavenger of superoxide anions, nor prostacyclin, an inhibitor of neutrophils, altered myocardial scar formation[61,62] while indomethacin, which does not inhibit leukocytes,[63] caused infarct expansion and thinning.[64,65] Thus, there is no clear relationship between a drug's effect on neutrophil function and the healing of infarcted myocardium.

FUTURE RESEARCH DIRECTIONS

Despite the growing evidence that the activation of neutrophils contributes to the ultimate extent of myocardial injury caused by ischemia and reperfusion, areas of uncertainty persist. First, do chemotactic stimuli and neutrophil activation occur soon enough after coronary artery occlusion to play an important role in the post-ischemic dysfunction, known as myocardial stunning, that occurs after reversible injury due to brief (<15 min) periods of ischemia? Secondly, do neutrophils influence the degree of damage caused by prolonged (>3 h) periods of ischemia? It is possible that the opportunity to modify myocardial injury by modulating neutrophil-mediated damage is confined to a limited range of coronary occlusion times. A third question is whether delayed inhibition of neutrophil function is beneficial? Simpson et al.[56] demonstrated a rebound of injury that occurs after short-term inhibition of neutrophils, but there is little data regarding the effects of agents administered after the onset of reperfusion. Delayed treatment, i.e. 40 min after reperfusion, with superoxide dismutase plus catalase was ineffective,[66] while administration of BW 755C, a dual inhibitor of the lipoxygenase and cyclooxygenase pathways of arachidonic acid metabolism, 30 min after reperfusion resulted in diminished myocardial injury in similar experiments.[20]

Both basic and clinical investigations of ischemic myocardial injury must recognize that there is emerging evidence for a component of oxygen radical production not attributable to neutrophils, for example, that incurred during asanguinous global ischemia and reperfusion.[67] The enzyme xanthine oxidase is one proposed source of non-leukocyte-derived oxygen radicals.[68] Additional sources must also exist, for crystalloid-perfused rabbit hearts exhibit oxygen radical signals during post-ischemic reflow[67] and display improved ventricular function when free radical scavengers are added to the perfusate[69] despite the lack of xanthine oxidase activity in rabbit hearts.[70,71] Clearly, further investigation is required to elucidate the pathogenesis of myocardial injury due to ischemia and reperfusion.

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REFERENCES

1. Grupo Italiano per lo studio della streptochinasi nell' infarto miocardico (GISSI). Effectiveness of intravenous thrombolytic treatment in acute myocardial infarction. *Lancet* **22** February; 1986.
2. ISIS Steering Committee. Intravenous streptokinase given within 0–4 hours of onset of myocardial infarction reduced mortality in ISIS-2. *Lancet* **28** February; 1987.
3. Braunwald, E.; Kloner, R. A. Myocardial reperfusion: A double-edged sword? *J. Clin. Invest.* **76**:1713–1719; 1985.
4. Cannon, R. O.; Butany, J. W.; McManus, B. M.; Speir, E.; Kravitz, A. B.; Bolli, R.; Ferrans, V. J. Early degradation of collagen after acute myocardial infarction in the rat. *Am. J. Cardiol.* **52**:390–395; 1983.
5. Monboisse, J. C.; Braquet, P.; Randoux, A.; Borel, J. P. Nonenzymatic degradation of acid-soluble calf skin collagen by superoxide ion: protective effect of flavonoids. *Biochem. Pharmacol.* **32**:53–58; 1983.
6. Bolli, R.; Cannon, R. O.; Speir, E.; Goldstein, R. E.; Epstein, S. E. Role of cellular proteinases in acute myocardial infarction. I. Proteolysis in nonischemic and ischemic rat myocardium and the effects of antipain, leupeptin, pepstatin and chymostatin administered in vivo. *J. Am. Coll. Cardiol.* **2**:671–680; 1980.
7. Bolli, R.; Cannon, R. O.; Speir, E.; Goldstein, R. E.; Epstein, S. E. Role of cellular proteinases in acute myocardial infarction. II. Influence of in vivo supression of myocardial proteolysis by antipain, leupeptin and pepstatin on myocardial infarct size in the rat. *J. Am. Coll. Cardiol.* **2**:681–688; 1983.
8. Apprill, P.; Schmitz, J. M.; Campbell, W. B.; Tilton, G.; Ashton, J.; Raheja, S.; Buja, L. M.; Willerson, J. T. Cyclic blood flow variations induced by platelet-activating factor in stenosed canine coronary arteries dispite inhibition of thromboxane synthetase, serotonin receptors, and adrenergic receptors. *Circulation* **72**:397–405; 1985.
9. Jackson, C. V.; Schumacher, W. A.; Kunkel, S. L.; Driscoll, E. M.; Lucchesi, B. R. Platelet-activating factor and the release of a platelet-derived coronary artery vasodilator substance in the canine. *Circ. Res.* **58**:218–229; 1986.
10. Gay, J. C.; Beckman, J. K.; Zaboy, K. A.; Lukens, J. N. Modulation of neutrophil oxidative responses to soluble stimuli by platelet-activating factor. *Blood* **67**:931–936; 1986.
11. Kenzora, J. L.; Perez, J. E.; Bergmann, S. R.; Lange, L. G. Effects of acetyl glyceryl ether of phosphorylcholine (Platelet activating factor) on ventricular preload, afterload, and contractility in dogs. *J. Clin. Invest.* **74**:1193–1203; 1984.
12. Sybertz, E. J.; Watkins, R. W.; Baum, T.; Pula, K.; Rivelli, M. Cardiac, coronary and peripheral vascular effects of acetyl glyceryl ether phosphoryl choline in the anesthetized dog. *J Pharmacol. Exp. Ther.* **232**:156–162; 1985.
13. Michelassi, F.; Castorena, G.; Hill, R. D.; Lowenstein, E.; Watkins, W. D.; Petkau, A. J.; Zapol, W. M. Effects of leukotrienes B4 and C4 on coronary circulation and myocardial contractility. *Surgery* **94**:267–275; 1983.

14. Ezra, D.; Boyd, L. M.; Feuerstein, G.; Goldstein, R. E. Coronary constriction by leukotriene C4, D4, and E4 in the intact pig heart. *Am. J. Cardiol.* **51**:1451–1454; 1983.
15. Bittl, J. A.; Pfeffer, M. A.; Lewis, R. A.; Mehrotra, M. M.; Corey, E. J.; Austen, K. F. Mechanism of the negative inotropic action of leukotrienes C4 and D4 on isolated rat heart. *Cardiovasc. Res.* **19**:426–432; 1985.
16. Roth, D. M.; Leffer, D. J.; Hock, C. E.; Lefer, A. M. Effects of peptide leukotrienes on cardiac dynamics in rat, cat, and guinea pig hearts. *Am. J. Physiol.* **249**:H477–H484; 1985.
17. Ford-Hutchinson, A. W. Leukotrienes: their formation and role as inflammatory mediators. *Fed. Proc.* **44**:25–29; 1985.
18. Gimbrone, M. A.; Brock, A. F.; Schafer, A. I. Leukotriene B4 stimulates polymorphonuclear leukocyte adhesion to cultured vascular endothelial cells. *J. Clin. Invest.* **74**:1552–1555; 1984.
19. Jolly, S. R.; Lucchesi, B. R. Effect of BW755C in an occlusion-reperfusion model of ischemic myocardial injury. *Am. Heart J.* **106**:8–13; 1983.
20. Mullane, K. M.; Read, N.; Salmon, J. A.; Moncada, S. Role of leukocytes in acute myocardial infarction in anesthetized dogs: relationship to myocardial salvage by anti-inflammatory drugs. *J. Pharmacol. Exp. Ther.* **228**:510–522; 1984.
21. Shea, M. J.; Murtagh, J. J.; Jolly, S. R.; Abrams, G. D.; Pitt, B.; Lucchesi, B. R. Beneficial effects of nafazatrom on ischemic reperfused myocardium. *Eur. J. Pharmacol.* **102**:63–70; 1984.
22. Bednar, M.; Smith, B.; Pinto, A.; Mullane, K. M. Nafazatrom-induced salvage of ischemic myocardium in anesthetized dogs is mediated through inhibition of neutrophil function. *Circ. Res.* **57**:131–141; 1985.
23. Fantone, J. C.; Ward, P. A. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am. J. Pathol.* **107**:397–418; 1982.
24. Weiss, S. J.; Lobuglio, A. F. Biology of disease: phagocyte-generated oxygen metabolites and cellular injury. *Lab. Invest.* **47**:5–18; 1982.
25. Werns, S. W.; Shea, M. J.; Lucchesi, B. R. Free radicals and myocardial injury: pharmacologic implications *Circulation* **74**:1–5; 1986.
26. Rowe, G. T.; Manson, N. H.; Caplan, M.; Hess, M. L. Hydrogen peroxide and hydroxyl radical mediation of activated leukocyte depression of cardiac sarcoplasmic reticulum. *Circ. Res.* **53**:584–591; 1983.
27. Gillespie, M. N.; Kojima, S.; Kunitoma, M.; Jay, M. Coronary and myocardial effects of activated neutrophils in perfused rabbit hearts. *J. Pharmacol. Exp. Ther.* **239**:836–840; 1986.
28. Varani, J.; Fligiel, S. E. G.; Till, G. O.; Kunkel, R. G.; Ryan, U. S.; Ward, P. A. Pulmonary endothelial cell killing by human neutrophils. *Lab. Invest.* **53**:656–663; 1985.
29. Scott, J. A.; Khaw, B. A.; Locke, E.; Haber, E.; Homcy, C. The role of free radical-mediated processes in oxygen-related damage in cultured murine myocardial cells. *Circ. Res.* **56**:72–77; 1985.
30. Friedman, B. J.; Jay, M.; Gillespie, M. N. Release of leukotriene B4 from hypoxic cultured chick myocardial cells. *Circulation* **74**:Suppl. II:II-348 abstr. (1986).
31. Hartmann, J. R.; Robinson, J. A.; Gunnar, R. M. Chemotactic activity in the coronary sinus after experimental myocardial infarction: effects of pharmacologic interventions on ischemic injury. *Am. J. Cardiol.* **40**:550–555; 1977.
32. Rossen, R. D.; Swain, J. L.; Michael, L. H.; Weakley, S.; Giannini, E.; Entman, M. L. Selective accumulation of the first component of complement and leukocytes in ischemic canine heart muscle. *Circ. Res.* **57**:119–130; 1985.
33. Rossen, R. D.; Reisberg, M. A.; Michael, L. H.; Moake, J. N.; Weakley, S.; Giannini, E.; Entman, M. L. Cardiac ischemia releases myocardial peptides which complex with the first component of complement. *Fed. Proc.* **45**:940 abstr. (1986).
34. Jennings, R. B.; Reimer, K. A. Factors involved in salvaging ischemic myocardium: effect of reperfusion of arterial blood. *Circulation* **68**:Suppl. I:I-25–I-36; 1983.
35. Mullane, K. M.; Kraemer, R.; Smith, B. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. *J. Pharmacol. Methods* **14**:157–167; 1985.
36. Romson, J. L.; Hook, B. G.; Kunkel, S. L.; Abrams, G. D.; Schork, M. A.; Lucchesi, B. R. Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* **67**:1016–1023; 1983.
37. Mitsos, S. E.; Askew, T. E.; Fantone, J. C.; Kunkel, S. L.; Abrams, G. D.; Schork, M. A.; Lucchesi, B. R. Protective effects of N-2-mercaptopropionyl glycine against myocardial reperfusion injury after neutrophil depletion in the dog: evidence for the role of intracellular-derived free radicals. *Circulation* **73**:1077–1086; 1986.
38. Jolly, S. R.; Kane, W. J.; Hook, B. G.; Abrams, G. D.; Kunkel, S. L.; Lucchesi, B. R. Reduction of myocardial infarct size by neutrophill depletion: effect of duration of occlusion. *Am. Heart J.* **112**:682–690; 1986.
39. Ksiezycka, E.; Hastie, R.; Maroko, P.R. Reduction in myocardial damage after experimental coronary artery occlusion by two techniques which deplete neutrophils (abstr). Circulation **68**:Suppl. III:III-185; 1983.
40. O'Neill, P. G.; Charlat, M. L.; Hartley, C. J.; Michael, L. H.; Roberts, R.; Bolli, R. Neutrophil depletion fails to attenuate postischemic myocardial dysfunction (abstr). *Circulation* **74**:Suppl. II:II-349; 1986.
41. Shea, M. J.; Simpson, P. J.; Werns, S. W.; Buda, A. J.; Alborzy-Khaghany, A. S.; Hoff, P. T.; Pitt, B.; Lucchesi, B. R. Effect of neutrophil depletion on recovery of "stunned" myocardium. (abstr). *Clin. Res.* **35**:327A abstr. (1987).
42. Engler, R.; Covell, J. W. Granulocytes cause post ischemic dysfunction following 15 minutes of myocardial ischemia (abstr). *Circulation* **74**:Suppl. II:II-373; 1986.
43. Myers, M. L.; Bolli, R.; Lekich, R. F.; Hartley, C. J.; Roberts, R. Enhancement of recovery of myocardial function by oxygen free-radical scavengers after reversible regional ischemia. *Circulation* **72**:915–921; 1985.
44. Przyklenk, K.; Kloner, R. A. Superoxide dismutase plus catalase improve contractile function in the canine model of the "stunned myocardium." *Circ. Res.* **58**:148–156; 1986.
45. Gross, G. J.; Farber, N. E.; Hardman, H. F.; Warltier, D. C. Beneficial actions of superoxide dismutase and catalase in stunned myocardium of dogs. *Am. J. Physiol* **250**:H372–H377; 1986.
46. Romson, J. L.; Bush, L. R.; Jolly, S. R.; Lucchesi, B. R. Cardioprotective effects of ibuprofen in experimental regional and global myocardial ischemia. *J Cardiovasc. Pharmacol.* **4**:187–196; 1982.
47. Bonow, R. O.; Lipson, L. C.; Sheehan, F. H.; Capurro, N. L.; Isner, J. M.; Roberts, W. C.; Goldstein, R. E.; Epstein, S. E. Lack of effect of aspirin on myocardial infarct size in the dog. *Am. J. Cardiol.* **47**:258–264; 1981.
48. Mullane, K. M.; McGiff, J. C. Platelet depletion and infarct size in an occlusion-reperfusion model of myocardial ischemia in anesthetized dogs. *J. Cardiovasc. Pharmacol.* **7**:733–738; 1985.
49. Jolly, S. R.; Schumacher, W. A.; Kunkel, S. L.; Abrams, G. D.; Liddicoat, J.; Lucchesi, B. R. Platelet depletion in experimental myocardial infarction. *Basic Res. Cardiol.* **80**:269–279; 1985.
50. Romson, J. L.; Hook, B. G.; Rigot, V. H.; Schork, M. A.; Swanson, D. P.; Lucchesi, B. R. The effect of ibuprofen on accumulation of indium-111-labeled platelets and leukocytes in experimental myocardial infarction. *Circulation* **66**:1002–1011; 1982.
51. Flynn, P. J.; Becker, W. K.; Vercellotti, G. M.; Weisdorf, D. J.; Craddock, P. R.; Hammerschmidt, D. E.; Lillehei, R. C.; Jacob, H. S. Ibuprofen inhibits granulocyte responses to inflammatory mediators. *Inflammation* **8**:33–44; 1984.
52. Jugdutt, B. I.; Hutchins, G. M.; Bulkley, B. H.; Becker, L. C. Dissimilar effects of prostacyclin, prostaglandin E1, and pros-

taglandin E2 on myocardial infarct size after coronary occlusion in conscious dogs. *Circ. Res.* **49**:685–700; 1981.
53. Melin, J. A.; Becker, L. C. Salvage of ischemic myocardium by prostacyclin during experimental myocardial infarction. *J. Am. Coll. Cardiol.* **2**:279–286; 1983.
54. Simpson, P. J.; Mitsos, S. E.; Ventura, A.; Gallagher, K. P.; Fantone, J. C.; Abrams, G. D.; Schork, M. A.; Lucchesi, B. R. Prostacyclin protects ischemic reperfused myocardium in the dog by inhibition of neutrophil activation. *Am. Heart J.* **113**:129–137; 1987.
55. Simpson, P. J.; Mickelson, J.; Fantone, J. C.; Gallagher, K. P.; Lucchesi, B. R. Iloprost inhibits neutrophil function in vitro and in vivo and limits experimental infarct size in the canine heart. *Circ. Res.* in press; 1987.
56. Simpson, P. J.; Mickelson, J.; Fantone, J. C.; Gallagher, K. P.; Lucchesi, B. R. Sustained myocardial protection by iloprost with prolonged infusion in a canine model of temporary regional ischemia. *Fed. Proc.* **46**:1144 abstr. (1987).
57. MacLean, D.; Fishbein, M. C.; Braunwald, E.; Maroko, P. R. Long-term preservation of ischemic myocardium after experimental coronary artery occlusion. *J. Clin. Invest.* **61**:541–551; 1978.
58. Hammerman, H.; Kloner, R. A.; Hale, S.; Schoen, F. J.; Braunwald, E. Dose-dependent effects of short-term methylprednisolone on myocardial infarct extent, scar formation, and ventricular function. *Circulation* **68**:446–452; 1983.
59. Bulkley, B. H.; Roberts, W. C. Steroid therapy during acute myocardial infarction. *Am. J. Med.* **56**:244–250; 1974.
60. Brown, E. J.; Kloner, R. A.; Schoen, F. J.; Hammerman, H.; Hale, S.; Braunwald, E. Scar thinning due to ibuprofen administration after experimental myocardial infarction. *Am. J. Cardiol.* **51**:877–882; 1983.
61. Jugdutt, B. I. Delayed effects of early infarct-limiting therapies on healing after myocardial infarction. *Circulation* **72**:907–914; 1985.
62. Werns, S. W.; Shea, M. J.; Vaporciyan, A.; Phan, S.; Abrams, G. D.; Buda, A. J.; Pitt, B.; Lucchesi, B. R. Superoxide dismutase does not cause scar thinning after myocardial infarction. *J. Am. Coll. Cardiol.* **9**:898–902; 1987.
63. Higgs, G. A.; Eakins, K. E.; Mugridge, K. G.; Moncada, S.; Vane, J. R. The effects of non-steroid anti-inflammatory drugs on leukocyte migration in carrageenin-induced inflammation. *Eur. J. Pharmacol.* **66**:81–86; 1980.
64. Hammerman, H.; Kloner, R. A.; Schoen, F. J.; Brown, E. J.; Hale, S.; Braunwald, E. Indomethacin-induced scar thinning after experimental myocardial infarction. *Circulation* **67**:1290–1295; 1983.
65. Hammerman, H.; Schoen, F. J.; Braunwald, E.; Kloner, R. A. Drug-induced expansion of infarct: morphologic and functional correlations. *Circulation* **69**:611–617; 1984.
66. Jolly, S. R.; Kane, W. J.; Bailie, M. B.; Abrams, G. D.; Lucchesi, B. R. Canine myocardial reperfusion injury. *Circ. Res.* **54**:277–285; 1984.
67. Zweier, J. L.; Flaherty, J. T.; Weisfeldt, M. L. Observation of free radical generation in the post-ischemic heart. *Circulation* **72**:Suppl. III:III-350 abstr. (1985).
68. McCord, J. M. Oxygen-derived free radicals in postischemic tissue injury. *N. Engl J. Med.* **312**:159–163; 1985.
69. Ambrosio, G.; Weisfeldt, M. L.; Jacobus, W. E.; Flaherty, J. T. Evidence for a reversible oxygen radical-mediated component of reperfusion injury: reduction by recombinant human superoxide dismutase administered at the time of reflow. *Circulation* **75**:282–291; 1987.
70. Grum, C. M.; Ragsdale, R. A.; Ketai, L. H.; Shlafer, M. Absence of xanthine oxidase or xanthine dehydrogenase in the rabbit myocardium. *Biochem. Biophys. Res. Commun.* **141**:1104–1108; 1986.
71. Grum, C. M.; Ketai, L. H.; Myers, C. L.; Shlafer, M. Purine efflux after cardiac ischemia: relevance to allopurinal cardioprotection. *Am. J. Physiol.* **252**:H368–H373; 1987.