

Review

The role of nitric oxide and cell adhesion molecules on the microcirculation in ischaemia–reperfusion

Allan M. Lefer^{a,*}, David J. Lefer^b

^a Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA

^b Department of Medicine, Cardiology Section, Tulane University School of Medicine, New Orleans, LA 70112, USA

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Abstract

The microcirculation undergoes a profound degree of endothelial dysfunction within minutes (i.e., 2.5 to 5 min) following reperfusion of ischaemic vasculature. This has been documented in the coronary and mesenteric microcirculation. The endothelial dysfunction is characterized by a loss in basal and agonist-mediated nitric oxide (NO) produced by the vascular endothelium. The loss of NO results in upregulation of cell adhesion molecules (CAMs) particularly P-selectin 10–20 min following reperfusion. Thus, CAM upregulation renders the endothelium sticky, and a marked degree of leukocyte adherence (particularly neutrophils) occurs 20 min following reperfusion. This enhanced involvement of neutrophils leads to neutrophil infiltration into the underlying tissue (e.g., myocardium) within 2–3 h of reperfusion. The infiltration of neutrophils leads to reperfusion injury (i.e., necrosis) which is significant at 3 h but becomes profound at 4.5 h following reperfusion. Cardiac necrosis can be significantly attenuated by treatment with NO, an organic NO donor, L-arginine, or specific blockers of CAMs given just prior to reperfusion. This approach is a promising one for a variety of types of reperfusion injury.

Keywords: Neutrophils; Nitric oxide; Endothelium; Cell adhesion molecules; Selectins; Reperfusion; Microcirculation

1. Introduction

Ischaemia is a marked reduction in the blood flow to an organ or a vascular bed. Ischaemia can lead to significant tissue injury and cell death if it is prolonged. Therefore, one of the major goals in the treatment of ischaemia is to restore blood flow to normal values (i.e., to “reperfuse” the ischaemic vascular bed). With the advent of thrombolytic agents (e.g., streptokinase, tPA) and percutaneous transluminal coronary angioplasty (PTCA), this has become accepted clinical practice in the case of myocardial ischaemia, as well as in mesenteric ischaemia. However, reperfusion has led to a new pathophysiological condition called “reperfusion injury”, a phenomenon which actually provokes a distinct degree of tissue injury due to the

process of abrupt reperfusion of the ischaemic vascular bed [1,2].

Reperfusion injury is an interesting phenomenon, since it is an unexpected occurrence, and has a complex pathophysiology [3,4]. Reperfusion injury involves an orchestrated sequence of cellular and molecular events, which have only recently been elucidated. Reperfusion injury develops over a time period of several hours (i.e., 2 to 24 h) and involves two very distinct but related events. The key events in this two-stage hypothesis of reperfusion injury have been coined “the endothelial trigger” and “the neutrophil amplification” steps [5]. We now know that a major early event leading to endothelial dysfunction [6] is the loss of endothelial cell release of nitric oxide (NO) [7] which occurs within 2.5 to 5 min following re-establishment of flow in either an intact animal subjected to myocardial or mesenteric [8] ischaemia or in an isolated perfused organ (i.e., heart) subjected to perfusion with a blood-free solution [9]. This endothelial dysfunction

* Corresponding author. Tel. (+1-215) 503-7760; Fax (+1-215) 503-2073.

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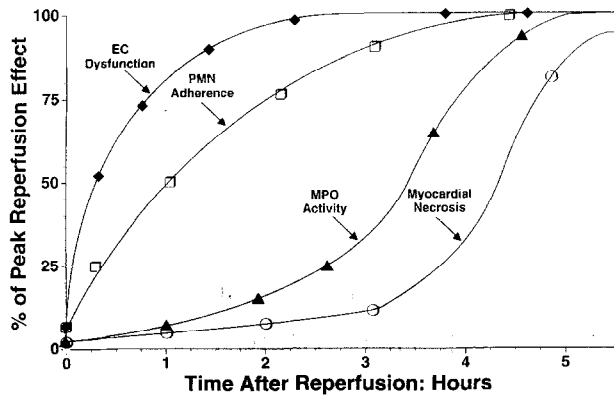


Fig. 1. Temporal relationships of key variables in myocardial ischaemia-reperfusion, including endothelial dysfunction (i.e., loss of NO) which occurs 2.5 to 5 min post-reperfusion, increased endothelial adhesiveness which occurs 20 min post-reperfusion, neutrophil infiltration (as assessed by cardiac myeloperoxidase (MPO) activity) which occurs 180 min post-reperfusion, and significant myocardial necrosis which occurs 270 min post-reperfusion.

is followed by a sequence of events which result from the altered status of the endothelium. The major second step in the process of reperfusion injury is the neutrophil amplification step, which involves leukocyte-endothelial interaction leading to neutrophil (PMN) adherence to the endothelium and transendothelial migration of neutrophils across the endothelium. In this manner, neutrophils can release their mediators while either in the microvasculature in which case these mediators can diffuse across the endothelium to injure parenchymal cells (e.g., cardiac myocytes, hepatocytes [10]), or the neutrophils can leave the microcirculation, migrate to and adhere to matrix proteins or to these cells [11]. Thus, there is a concerted effort resulting in a well characterized interaction between the activated neutrophil and the dysfunctional endothelium resulting in reperfusion injury [12]. This is the major theme of this review. Fig. 1 illustrates the major temporal relationships of these seminal events in reperfusion injury as they relate to the ischaemic-reperfused myocardium.

Clearly, one of the earliest pathophysiologic events in the reperfusion process is endothelial cell (EC) dysfunction characterized by a marked reduction in EC release of NO which occurs as early as 2.5 to 5 min post-reperfusion. This is followed by a dramatic increase in PMN adherence to the reperfused endothelium, becoming highly significant at 20 min post-reperfusion. This enhanced leukocyte adherence to the endothelium leads to capillary plugging and edema formation resulting in a reduction in coronary blood flow [3,13]. This response to ischaemia and reperfusion is termed the "no-reflow" phenomenon. Following PMN adherence to the endothelium, transendothelial migration of activated PMNS can occur with significant increases in PMN accumulation in the myocardium, as determined by elevated myeloperoxidase (MPO) activity at 3–6 h post-reperfusion. Increased MPO activity is indicative of neutrophil infiltration since MPO is found almost exclusively

within neutrophils [14]. These events culminate in a profound degree of myocardial cell necrosis at 4 to 24 h post-reperfusion. The major goal of this paper is to review and discuss the relevant mechanisms for this sequence of events as they occur within the microcirculation, and to integrate these phenomena into a sequential pathway which can largely explain the phenomenon of reperfusion injury.

2. Role of cell adhesion molecules (CAMs) in reperfusion injury

There are three major families of cell adhesive molecules which regulate leukocyte-endothelial cell interaction. These are: (a) the selectins (i.e., P-selectin, L-selectin, E-selectin) [15,16], (b) the $\beta 2$ integrins (e.g., CD11/CD18) [17], and (c) the immunoglobulin superfamily [18] (e.g., ICAM-1, VCAM-1, PECAM-1).

The selectins are a family of glycoproteins that appear to be involved in the early interaction between leukocytes and the endothelium. L-selectin is constitutively present on the surface of leukocytes (particularly neutrophils and lymphocytes) and participates in early leukocyte rolling on the activated or inflamed endothelium. P-selectin is found in Weibel-Palade bodies of endothelial cells and α -granules of platelets, and can be rapidly up-regulated and exteriorized to the cell surface by humoral activators including thrombin, histamine, hydrogen peroxide and complement fragments. P-selectin is maximally upregulated in 10–20 min [19,20]. These, and other data strongly implicate P-selectin as a major mediator of leukocyte rolling. E-selectin is expressed on the surface of endothelial cells, and is more slowly upregulated by cytokines including IL-1 β and TNF- α via a process requiring de novo protein synthesis [21]. E-selectin expression is not significantly upregulated until 4–6 h, and thus is not a prominent feature of the acute phase of reperfusion injury. E-selectin may however be involved in later phenomena of reperfusion injury [22,23].

The $\beta 2$ integrins comprise an important group of heterodimeric glycoproteins present in leukocytes. The three major members of this group are CD11a/CD18, CD11b/CD18, and CD11c/CD18. Although all three of these $\beta 2$ integrins may play important roles in adhesive interactions between different cell types, CD11b/CD18, also known as Mac-1 or Mo1 appears to be the major form responsible for firm adhesion of neutrophils to the vascular endothelium. When L-selectin is shed from the neutrophil pseudopods, CD11b/CD18 is rapidly upregulated and triggers firm adhesion of PMNs to the vascular endothelium.

Among the immunoglobulin superfamily members, intercellular adhesion molecule-1 (ICAM-1) is an important mediator in the firm adhesion of neutrophils to the vascular endothelium. It appears to be the major counter-receptor on endothelial cells for CD11/CD18. ICAM-1 is constitutively present on the surface of endothelial cells to a

moderate degree, and can be strongly upregulated over a period of 2–4 h by cytokines including IL-1 β , IL-8, and TNF- α . Another member of this immunoglobulin superfamily is platelet–endothelial cell adhesion molecule-1 or PECAM-1 which is constitutively present on the surface of platelets, leukocytes, and endothelial cells [24,25]. On endothelial cells, it is concentrated at the intercellular junctions, and is thought to be intimately involved in the regulation of transendothelial migration of leukocytes [25,26].

Thus, there are a number of cell adhesion molecules which act in a concerted manner to influence the interaction between freely flowing leukocytes in the microcirculation, progressing through a slowing down phase (or “rolling”) to an arrested phase (or firm adherence) to an activated phase, and finally to a transported phase (or transendothelial migration) [27]. Although these steps are important in combatting localized infections in terms of bringing phagocytic leukocytes (e.g., neutrophils) to the site of the infection, these same processes are inappropriate in a local inflammatory state such as that existing at the site of reperfusion of an ischaemic heart. This has led to an intensive investigation of the role of specific adhesion molecules in this process. These investigations have been facilitated by a variety of specific monoclonal or polyclonal antibodies, each directed against a specific CAM. Table 1 summarizes the effects of adhesion molecule antibodies on PMN activity and tissue injury in acute myocardial ischaemia–reperfusion.

The inhibition of CAMs with monoclonal antibodies leads to a marked preservation of post-ischaemic myocardial blood flow in dogs subjected to regional myocardial ischaemia and reperfusion. A recent study [37] utilizing 15 μ m radiolabelled microspheres precisely measured blood in the coronary circulation following 90 min of coronary ischaemia and 4.5 h of reperfusion. The results of this study are summarized in Fig. 2 and reveal that monoclonal antibodies directed against P-selectin and ICAM-1 significantly preserve post-ischaemic transmural myocardial blood flow. Thus, interference with leukocyte “rolling”

Table 1
Effects of specific antibodies against CAMs in myocardial ischaemia–reperfusion

Antibody against adhesion molecule	Level of protection		
	Inhibition of PMN adherence	Diminished PMN infiltration	Reduced tissue injury
CD18 [28,29]	+++	+++	+++
CD11 α [30]	NM	++	++
P-selectin [31,32]	+++	+++	+++
L-selectin [33,34]	+++	+++	+++
E-selectin [35]	0	0	0
ICAM-1 [35–37]	+++	+++	+++
PECAM-1 [38,39]	0	+++	+++

0 = no effect; + = small effect; ++ = moderate effect; +++ = large effect; NM = not measured.

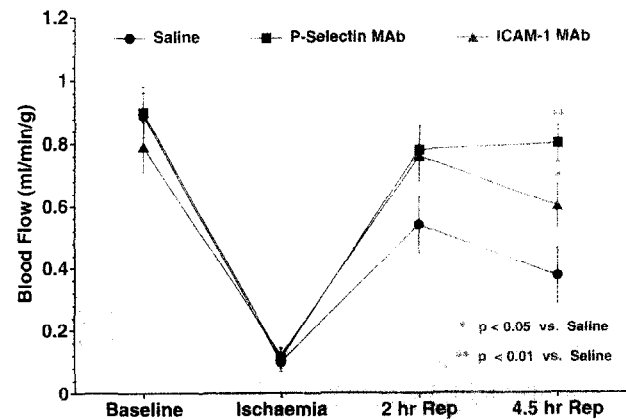


Fig. 2. Transmural myocardial blood flow (ml/min per g) as determined with radiolabelled 15 μ m microspheres in dogs subjected to 90 min of coronary ischaemia and reperfusion. Treatment with either an anti-P-selectin monoclonal antibody (PB1.3) or an anti-ICAM-1 monoclonal antibody (CL 18/6) significantly reduces the extent of microvascular “no-reflow” following ischaemia and reperfusion.

(P-selectin mediated) or inhibition of firm leukocyte adhesion (ICAM-1 mediated) reduces the degree of microvascular dysfunction and dramatically improves post-ischaemic myocardial blood flow.

From these results, it is apparent that interfering with leukocyte–endothelial interactions exerts a salutary effect on the reperfusion injury occurring following reperfusion of the ischaemic coronary vasculature. If one inhibits leukocyte rolling, the earliest step in the WBC–endothelial cascade, by blocking either L-selectin or P-selectin, one can achieve a significant degree of cardioprotection. Moreover, it is also clear that interference with the process of neutrophil adherence to the endothelium is of paramount importance in attenuating reperfusion injury, although blocking PMN migration into ischaemic tissue as is the case with PECAM-1 blockade can also be fruitful [38,39].

Thus far, E-selectin does not appear to play a key role in early acute reperfusion injury since it probably is not activated until 4–6 h, and then perhaps not by the stimuli occurring in reperfusion injury [20,40]. However, it may be important in later injury [22,40].

Monoclonal antibodies are useful tools, but in their usual form (e.g., murine anti-human antibodies) they are not practical as therapeutic agents in humans. Therefore, several alternative strategies have emerged. One such approach is to humanize the monoclonal antibody by a process of restructuring the complementary-determining regions of the murine antibody with the respective human immune system as a foreign protein. This has been done in the case of the anti-L-selectin monoclonal antibody (DREG-200) [34]. Comparable results were obtained with humanized DREG-200 as with murine DREG-200 in myocardial reperfusion injury [33,34]. This may be a useful strategy applicable to other monoclonal antibodies. An-

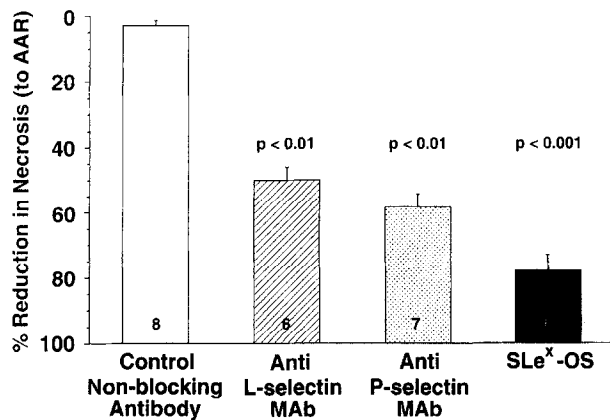


Fig. 3. Comparison of different forms of anti-selectin therapy in acute myocardial ischaemia-reperfusion in cats. Use of a monoclonal antibody directed against either P-selectin or L-selectin attenuates the area-of-necrosis indexed to the area-at-risk (AAR) by 50 to 55%, whereas a sialyl Lewis^x-oligosaccharide, which blocks all the selectins, attenuates the necrosis by 75 to 80%.

other strategy is to develop small molecule antagonists of CAMs based on analogs of their ligands. This has been done successfully in the case of analogs of sialyl Lewis^x, a major ligand for the three selectin family members [41]. This SLe^x-oligosaccharide (SLe^x-OS) proved to be even more cardioprotective than any of the anti-selectin monoclonal antibodies [42,43]. This can be seen in Fig. 3.

This remarkable degree of cardioprotection by SLe^x-OS was achieved at a dose of 10 mg/kg given intravenously. However, this carbohydrate is difficult and expensive to synthesize, and has a half-life in blood of 15–30 min [42,43]. One solution to these problems is to encapsulate this sugar in lipid particles (i.e., liposomes). Liposome-conjugated SLe^x-OS was found to protect to the same extent as conventional SLe^x-OS, but at a dose of only 400 µg/kg, a dose which is one-twenty-fifth that employed with the conventional SLe^x-OS [44]. This development may render the small molecule approach more feasible as a therapeutic agent in ischaemia-reperfusion.

Clearly, CAMs play a pivotal role in the pathogenesis of reperfusion injury, and a number of CAMs operate in several stages of leukocyte-endothelial interaction in the microvasculature. The major site of all of these interactions is the venular endothelium and, to a lesser extent, the arteriolar endothelium. Moreover, similar relationships occur in other microvasculatures, notably the mesenteric microcirculation [45–47].

Further work is necessary to determine in other inflammatory states whether these interventions will be effective, at what dose, and during what time duration. In this regard, SLe^x-OS has also been found to be effective in traumatic shock in rats [45]. This broadens the base of effectiveness of anti-selectin therapy to other species and other circulatory disorders. Thus, blockade of CAMs may prove to be useful in a variety of related disease states.

3. Nitric oxide in ischaemia-reperfusion

As discussed in the first section of this review, one of the earliest signs of endothelial dysfunction following reperfusion of an ischaemic vascular bed (e.g., coronary, mesenteric vasculature) is a decreased endothelial release of NO. This is true both for agonist-mediated release of NO (e.g., vasorelaxation in response to endothelium-dependent vasodilators) and for basal NO release (e.g., vasoconstriction response to NOS inhibitors) [48]. The reduced release of NO from the ischaemic-reperfused endothelium measured by either method occurs 2.5 to 5 min post-reperfusion, persists for hours, and appears to be related to superoxide radicals produced by the abrupt reoxygenation of the reperfusion process [7,9,48]. Thus, a component of the reduced NO release is due to enhanced quenching of NO by superoxide radicals.

Since reduced NO release is a marked and early event in reperfusion injury, one approach to treating the consequences of reperfusion injury is early replacement of the lost or reduced NO. This was first attempted in acute myocardial ischaemia-reperfusion by Johnson et al. [49] who infused NO gas dissolved in aqueous media into cats subjected to acute myocardial ischaemia-reperfusion. The NO was dissolved in O₂-purged saline or distilled water, and was bioassayed on isolated aortic rings. The amount of NO infused was titrated to give an amount of NO just at the threshold of vasorelaxation (i.e., that concentration of NO which reduced arterial blood pressure only 5 to 10 mmHg). This was calculated to a concentration of NO of about 10–20 nM in the microcirculation [49]. The result of this NO infusion on subsequent myocardial necrosis is shown in Fig. 4.

This NO infusion therapy was repeated in mesenteric ischaemia-reperfusion with comparable results [50]. Cats receiving NO developed a much more moderate degree of circulatory shock than those cats receiving control solu-

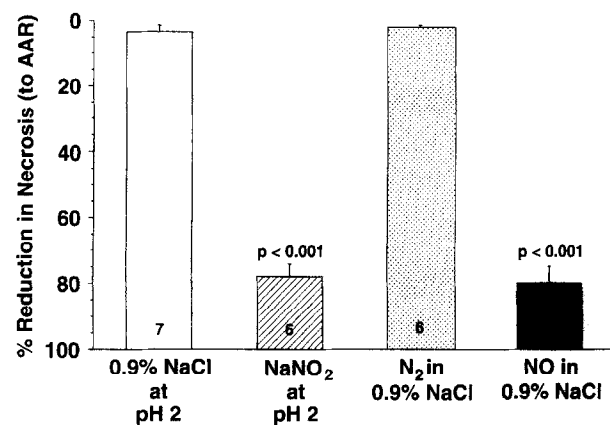


Fig. 4. Use of nitric oxide gas dissolved in aqueous medium infused just before reperfusion in a feline model of myocardial ischaemia-reperfusion. NO therapy was very effective in preventing myocardial necrosis within the area-at-risk (AAR) compared to a solution lacking NO.

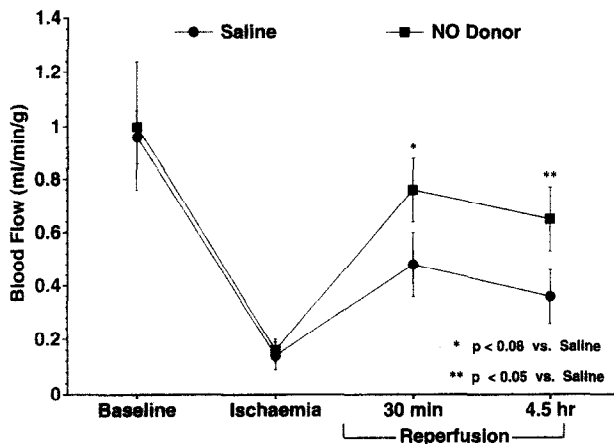


Fig. 5. Myocardial blood flow in canine hearts following 90 min of ischaemia and reperfusion for 4.5 h. Intracoronary treatment with the NO donor, CAS-1609, significantly improved transmural blood flow compared to saline at 4.5 h of reperfusion indicating that NO therapy is beneficial in the ischaemic–reperfused myocardium.

tions [50]. Since that time, a variety of NO replacement strategies have been utilized including inorganic (e.g., NaNO_2) and organic NO donors, and the amino acid substrate for NO biosynthesis, L-arginine, in ischaemia–reperfusion. Not all NO donors are equivalent since they all have different rates of NO release, and some release other substances (e.g., SIN-1 releases superoxide radicals). Table 2 summarizes the results of NO replacement therapy in acute myocardial ischaemia–reperfusion.

Since NO inhibits PMN and platelet adhesion and is also a potent vasodilator, it would seem plausible that NO therapy in the ischaemic–reperfused heart would substantially attenuate the “no reflow” phenomenon induced by microvascular plugging with leukocytes. Indeed, this is the case as observed by Pabla et al. [55] in a recent experimental study of ischaemic–reperfused dog hearts treated with a long-acting NO-donating compound, CAS-1609. The effects of this novel NO donor on post-ischaemic blood flow are summarized in Fig. 5. Low dose, intracoronary administration of the NO donor maintained transmural myocardial blood flow at levels very similar to the pre-ischaemic baseline level throughout the reperfusion period. This dramatic effect of NO administration appears to be related to a reduction in PMN adhesion to the microvascular en-

dothelium as myocardial PMN accumulation was dramatically reduced in this study. Additionally, it is possible that the NO released by the NO donor could directly induce some coronary vasodilation.

The overall conclusion drawn from these studies is that NO supplementation either as authentic NO gas, a nitric oxide donor which releases NO in solution, or a precursor metabolite which metabolically leads to NO formation, can protect in ischaemia–reperfusion states against reperfusion injury. The one caveat here is that the dose of NO given must be carefully titrated so that systemic blood pressure does not decrease markedly, a side effect which would lead to systemic hypotension and possibly a “coronary steal” syndrome, either of which would defeat the purpose of the NO replacement. It is worth noting that the low levels of NO resulting from the NO replacement in addition to not exerting a significant vasodilator effect, also do not significantly depress myocardial contractility [58].

Since low levels of NO do not exert any significant direct haemodynamic effect, what is the basis of the cardioprotective effect of NO? The answer resides in a variety of cytoprotective actions of NO, the major ones being inhibition of platelet activation [59], inhibition of neutrophil adherence and activation [60,61], and scavenging of superoxide radicals [62,63]. Of these, the major effect in ischaemia–reperfusion appears to be directed against adherence of neutrophils to the vascular endothelium. This brings us back to the cell adhesion molecules. The next section of this review will discuss the interesting relationships between NO and CAMs as they relate to ischaemia–reperfusion.

4. Interrelationships between nitric oxide and cell adhesion molecules in ischaemia–reperfusion

For several years, evidence has been accumulating that NO modulates leukocyte–endothelial cell interactions, and may actually down-regulate specific cell adhesion molecules. The initial observation was made by McCall et al. [64] who showed that NO inhibits rat PMN aggregation by formyl-methionyl-leucyl-phenylalanine (fMLP). The next major observation which promoted this line of thought

Table 2
Nitric oxide (NO) replacement therapy in myocardial ischaemia–reperfusion

Form of NO replacement		Level of protection		
		Inhibition of PMN adherence	Diminished PMN infiltration	Reduced tissue injury
NO gas [49]		NM	++	+++
NO donor	NaNO_2 [51]	NM	+++	+++
	SPM-5185 [52,53]	++	++	+++
	SIN-1 [54]	++	0	++
	CAS-754 [55]	++	+	++
	CAS-1609 [56]	NM	+++	+++
L-arginine [57,58]		++	++	+++

0 = no effect; + = small effect; ++ = moderate effect; +++ = large effect; NM = not measured.

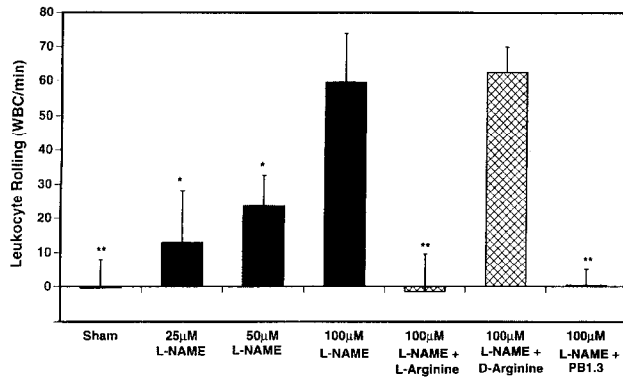


Fig. 6. Inhibition of nitric oxide synthase with L-NAME and leukocyte rolling along the venular endothelium in rats. The concentration-dependent increase in leukocyte rolling was overridden by addition of L-arginine but not by D-arginine indicating a NO-specific effect. An antibody directed against P-selectin (PB1.3) also blocked rolling. Thus, NO protects against upregulation of P-selectin on the vascular endothelium, thereby preventing leukocyte rolling, the prelude to leukocyte adhesion. (Modified from [61]).

was the pioneering work of Kubes et al. [60] who showed that superfusion of a NOS inhibitor in the cat mesenteric microvasculature resulted in a marked increase in adherence of leukocytes to the venular endothelium. These investigators also showed that this enhanced adhesiveness could be overcome by addition of a monoclonal antibody directed against CD18, the major neutrophil adhesion molecule responsible for firm cellular adhesion.

Additional evidence has been obtained in the coronary vasculature. In a series of studies employing nitric oxide

donors, Siegfried and coworkers [54] showed that several different NO donors attenuated cat neutrophil release of superoxide radical resulting in a NO-sparing effect on the coronary endothelium and on the mesenteric vascular endothelium [65]. This has been subsequently confirmed in human neutrophils for superoxide [66,67] and for hydrogen peroxide [68]. Although these data strongly point toward a marked anti-neutrophil effect of NO, they do little to associate the leukocyte-endothelial interaction with specific cell adhesion molecules.

More recently, efforts were made to ascertain which cell adhesion molecules were down-regulated by nitric oxide. A recent study [69] employing human aortic endothelial cells (HAECs) in culture found that NO donors or the NO precursor, L-arginine decreased basal ICAM-1 surface expression.

The interrelationships between NO and cell adhesion molecules and the relevance of these relationships to ischaemia-reperfusion were carefully studied by Davenpeck and coworkers [47,61,70]. These investigators employed intravital microscopy of the rat mesenteric microvasculature to further investigate the interactions between NO and CAMs. In the first of these three studies [47], these workers found that ischaemia-reperfusion of the mesenteric circulation, a condition which has been shown to dramatically reduce endothelial NO, resulted in a rapid increase in leukocyte "rolling" and adherence to the venular endothelium within the first 30 min following reperfusion. Moreover, immunohistochemistry of the intestinal microcirculation indicated a marked upregulation

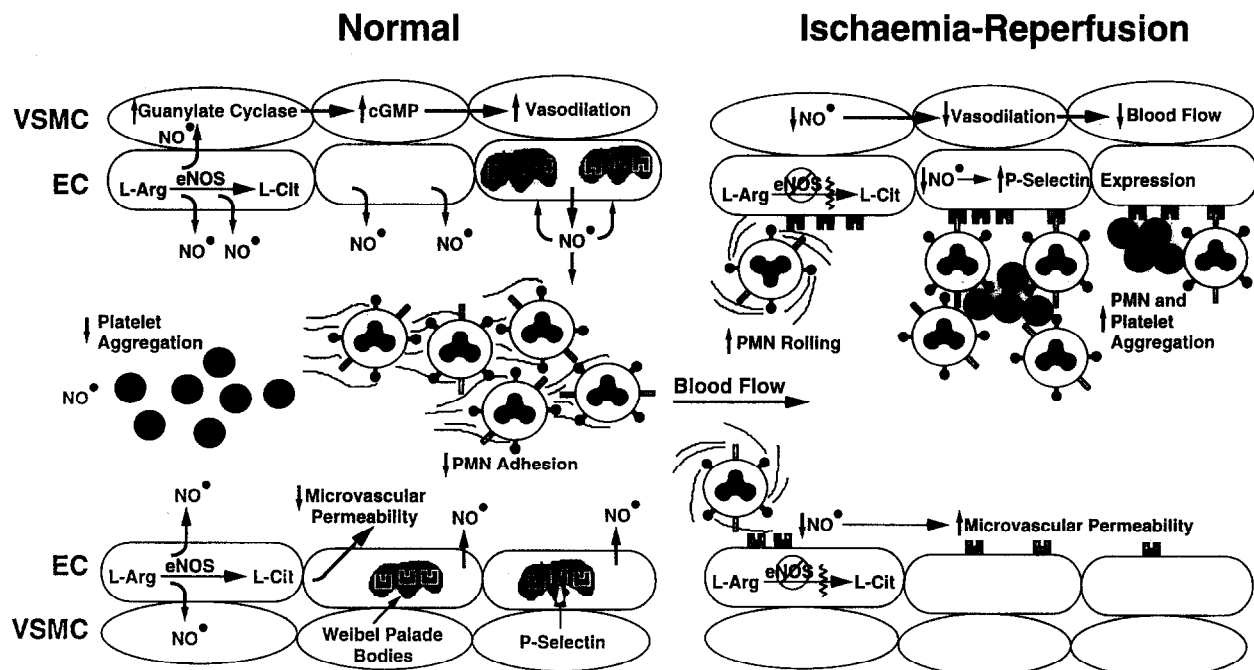


Fig. 7. Schematic diagram of the microvasculature showing the diverse beneficial effects of NO on microvascular homeostasis under normal conditions, and its relationships to endothelial cell P-selectin expression in the setting of ischaemia and reperfusion. L-arg = L-arginine, L-cit = L-citrulline, eNOS = endothelial nitric oxide synthase, PMN = neutrophil, EC = endothelial cell, VSMC = vascular smooth muscle cell.

of P-selectin expression on the venular endothelial surface 30 min post-reperfusion. These results clearly indicate that at a time when NO levels are known to be critically reduced, P-selectin is markedly upregulated at the endothelial site of enhanced leukocyte adherence, possibly due to increased superoxide and hydrogen peroxide levels.

These studies were followed closely by other findings implicating NO in these processes. Gauthier et al. [70] showed that in the same model of ischaemia–reperfusion of the rat mesenteric circulation, infusion of a NO donor (i.e., S-nitrosylacetyl-penicillamine, SNAP) markedly decreased post-reperfusion rolling and adherence of leukocytes on the venular endothelium. Moreover, infusion of SNAP concomitantly prevented P-selectin expression on the surface of the venular endothelium. In fact, SNAP infusion ameliorated the signs of circulatory shock usually occurring in mesenteric ischaemia–reperfusion. These results related the adhesive events to a clear lack of NO.

In the third study of this group, these results were extended and the full expression of this feedback loop was elucidated. Again utilizing the rat mesenteric microvasculature, Davenpeck et al. [61] discovered that superfusion of rat mesenteric loops with either of the NOS inhibitors L-NAME or L-NMMA mimicked the effects of ischaemia–reperfusion. Most of the studies were conducted with L-NAME. Fig. 6 illustrates some of the key findings with this NOS inhibitor on leukocyte rolling.

In the quiescent microcirculation, very little leukocyte rolling occurs 30–60 min following careful isolation of the rat mesentery. However, addition of 25 μM , 50 μM and 100 μM L-NAME resulted in a concentration-dependent increase in leukocyte rolling. This marked increase in rolling in the presence of 100 μM L-NAME could be overcome with equimolar concentrations of L-arginine but not of D-arginine, indicating that the increased leukocyte rolling induced by L-NAME is due to a decrease in NO levels rather than some non-specific effect of L-NAME. Moreover, leukocyte rolling could be completely abolished by administration of a monoclonal antibody directed against P-selectin. These findings were strengthened by immunohistochemical results showing that L-NAME upregulated P-selectin expression on the mesenteric microvascular endothelium, and that L-arginine suppressed that process. Moreover, similar results were obtained with leukocyte adherence. Thus, both leukocyte rolling and leukocyte adherence followed the same pattern of response, both being upregulated by decreased NO levels and downregulated by increased NO.

Furthermore, addition of either human recombinant superoxide dismutase (rhSOD) or 8-bromo-cyclic guanosine monophosphate (8-br-cGMP) could also attenuate the leukocyte–endothelial interactions [61]. These data clearly point to a dynamic interaction between oxygen-derived free radicals and P-selectin, perhaps by hydrogen peroxide upregulating P-selectin. Normally nitric oxide acts as a physiologic inhibitor of this process, attenuating adhesive

interactions between leukocytes and the vascular endothelium, but in situations where there is an acute increase in oxidant stress, endothelial cell generation of NO is dramatically compromised. Following attenuated NO release, P-selectin expression on the endothelium is upregulated and leukocyte rolling along the endothelium is markedly enhanced.

5. Summary

In summary, this review has focused on the phenomena of leukocyte–endothelial interaction in ischaemia–reperfusion and the mechanisms underlying these events. Particular emphasis has been placed on the key roles of cell adhesion molecules, particularly P-selectin in these processes, and the important interaction between nitric oxide generated by the vascular endothelium and these cell adhesion molecules. Fig. 7 gives a schematic illustration of these relationships.

References

- [1] Braunwald E, Kloner RA. Myocardial reperfusion: a double edged sword. *J Clin Invest* 1985;76:1713–1719.
- [2] Farb A, Kolodgie FD, Jenkins M, Virmani R. Myocardial infarct extension during reperfusion after coronary artery occlusion: pathologic evidence. *J Am Coll Cardiol* 1993;21:1245–1253.
- [3] Engler RL, Dahlgren MD, Morris DD, Paterson MA, Schmid-Schonbein AW. Role of leukocytes in response to acute myocardial ischemia reflow in dogs. *Am J Physiol* 1986;251:H314–H323.
- [4] Barroso-Aranda J, Schmid-Schonbein GW, Zweifach BW, Engler RL. Granulocytes and no-reflow phenomena in irreversible hemorrhagic shock. *Circ Res* 1988;63:437–447.
- [5] Bulkley GB. Mediators of splanchnic organ injury: overview and perspective. In: Marston A, Bulkley GB, Fiddian-Green RG, Haglund UH, eds. *Splanchnic Ischemia and Multiple Organ Failure*. London: Edward Arnold, 1989:183–194.
- [6] Mehta JL, Nichols WW, Donnelly WH, Lawson DL, Saldeen TGP. Acetylcholine and bradykinin after occlusion–reperfusion. *Circ Res* 1989;64:43–54.
- [7] Tsao PS, Aoki N, Lefer DJ, Johnson G, Lefer AM. Time course of endothelial dysfunction and myocardial injury during myocardial ischemia and reperfusion in the cat. *Circulation* 1990;82:1402–1412.
- [8] Lefer AM, Ma X-L. Endothelial dysfunction in the splanchnic circulation following ischemia and reperfusion. *J Cardiovasc Pharmacol* 1991;17(Suppl. 3):S186–S190.
- [9] Tsao PS, Lefer AM. Time course and mechanism of endothelial dysfunction in isolated ischemic and hypoxic perfused rat hearts. *Am J Physiol* 1990;259:H1660–H1666.
- [10] Buerke M, Weyrich AS, Lefer AM. Isolated cardiac myocytes are sensitized by hypoxia–reoxygenation to neutrophil released mediators. *Am J Physiol* 1994;266:H128–H136.
- [11] Smith CW, Entman ML, Lane CL, Beaudet AL, Ty TI, Youker K, Hawkins HK, Anderson DC. Adherence of neutrophils to canine cardiac myocytes in vitro is dependent on intercellular adhesion molecule-1. *J Clin Invest* 1991;88:1216–1223.
- [12] Lefer AM, Tsao PS, Lefer DJ, Ma X-L. Role of endothelial dysfunction in the pathogenesis of reperfusion injury following myocardial ischemia. *FASEB J* 1991;5:2029–2034.

- [13] Engler RL, Schmid-Schonbein GW, Pavlec RS. Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. *Am J Pathol* 1983;111:98–111.
- [14] Mullane KM, Kraemer R, Smith B. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. *J Pharmacol Meth* 1985;14:157–167.
- [15] Bevilacqua M, Nelson RM. Selectins. *J Clin Invest* 1993;91:379–387.
- [16] Lefer AM, Weyrich AS, Buerke M. Role of selectins, a new family of adhesion molecules, in ischaemia–reperfusion injury. *Cardiovasc Res* 1994;28:289–294.
- [17] Ruoslahti E. Integrins. *J Clin Invest* 1991;87:1–5.
- [18] Springer TA. Adhesion receptors of the immune system. *Nature* 1990;346:425–434.
- [19] McEver RP. GMP-140: a receptor for neutrophils and monocytes on activated platelet and endothelium. *J Cell Biochem* 1991;45:156–161.
- [20] Weyrich AS, Buerke M, Albertine KH, Lefer AM. Time course of coronary vascular endothelial adhesion molecule expression during reperfusion of the ischemic feline myocardium. *J Leukocyte Biol* 1995;57:45–55.
- [21] Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gibbrone MA. Identification of an inducible endothelial–leukocyte adhesion molecule. *Proc Natl Acad Sci USA* 1987;84:9238–42.
- [22] Lefer DJ, Flynn DM, Jeffords PR, Vo KD, Buda AJ. A sialyl Lewis^x-containing carbohydrate reduces infarct size following myocardial ischemia and prolonged reperfusion. *Circulation*, 1995;92:1-712.
- [23] Kukielka GL, Lanek CL, Manning AM, Michael LH, Entman ML, Smith CW, Anderson DC. Induction of myocardial ELAM-1 by ischemia and reperfusion. (Abstract) *FASEB J* 1993;7:A1060.
- [24] Albelda SM, Muller WA, Buck CA, Newman PJ. Molecular and cellular properties of PECAM-1 (endoCAM/CD31): a novel vascular cell–cell adhesion molecule. *J Cell Biol* 1991;114:1059–1068.
- [25] Muller WA, Weigl SA, Deng X, Phillips DM. PECAM-1 is required for transendothelial migration of leukocytes. *J Exp Med* 1993;178:449–460.
- [26] Vaporciyan AA, DeLisser HM, Yan HC, Mendiguren II, Thomas SR, Jones ML, Ward PA, Albelda SM. Involvement of platelet–endothelial cell adhesion molecule-1 in neutrophil recruitment in vivo. *Science (Wash. DC)* 1993;262:1580–15892.
- [27] Butcher EC. Leukocyte–endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 1991;67:1033–1036.
- [28] Ma X-L, Tsao PS, Lefer AM. Antibody to CD-18 exerts endothelial and cardiac protective effects in myocardial ischemia and reperfusion. *J Clin Invest* 1991;88:1237–1243.
- [29] Winquist R, Frei P, Harrison P, McFarland M, Letts G, Van G, Andrews L, Rothlein R, Hintze T. An anti-CD-18 Mab limits infarct size in primates following ischemia and reperfusion. *Circulation* 1990;82:III-701a (Abstract).
- [30] Simpson PJ, Todd RF, Fantone JC, Mickelson JK, Griffin JD, Lucchesi BR. Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (anti-Mo1, anti-CD11b) that inhibits leukocyte adhesion. *J Clin Invest* 1988;81:624–629.
- [31] Weyrich AS, Ma X-L, Lefer DJ, Albertine KH, Lefer AM. In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. *J Clin Invest* 1993;91:2620–2629.
- [32] Lefer DJ, Flynn DM, Buda AJ. Effects of a monoclonal antibody directed against P-selectin after myocardial ischemia and reperfusion. *Am J Physiol* 1996; 270: H88–H98.
- [33] Ma X-L, Weyrich AS, Lefer DJ, Buerke M, Albertine KH, Kishimoto TK, Lefer AM. Monoclonal antibody to L-selectin attenuates neutrophil accumulation and protects ischemic reperfused cat myocardium. *Circulation* 1993;88:649–658.
- [34] Buerke M, Weyrich AS, Murohara T, Queen C, Klingbeil CK, Co MS, Lefer AM. Humanized monoclonal antibody DREG-200 directed against L-selectin protects in feline myocardial reperfusion injury. *J Pharmacol Exp Ther* 1994;271:134–142.
- [35] Winquist RJ, Van LG, Andrews GY, Rothlein LK, Dreyer B, Smith WJ, Hintze CW. Monoclonal antibody to intercellular adhesion molecule-1, but not to endothelial–leukocyte adhesion molecule-1, protects against myocardial ischemia/reperfusion damage in anesthetized monkeys. *Circulation* 1992;86:I-79(abstract).
- [36] Ma X-L, Lefer DJ, Lefer AM, Rothlein R. Coronary endothelial and cardiac protective effects of a monoclonal antibody to intercellular adhesion molecule-1 in myocardial ischemia and reperfusion. *Circulation* 1992;86:937–946.
- [37] Lefer DJ, Flynn DM, Jeffords PR, Buda AJ. Combined inhibition of P-selectin and ICAM-1 reduces neutrophil accumulation and preserves coronary blood flow in the postischemic myocardium. *J Invest Med* 1995;43:312A (abstract).
- [38] Gumina RJ, Schultz J, Yao Z, Kenny D, Warltier DC, Gross G, Newman PJ. Antibody to PECAM-1 reduces myocardial infarct size. *J Invest Med* 1995;43(Suppl 2):312A.
- [39] Murohara T, Delyani JA, Albelda SM, Lefer AM. Blockade of platelet endothelial cell adhesion molecule-1 protects against myocardial ischemia and reperfusion injury in cats. *J Immunol* 1996;156:3550–3557.
- [40] Redl H, Dinges HP, Buurman WA, et al. Expression of endothelial leukocyte adhesion molecule-1 in septic but not traumatic/hypovolemic shock in the baboon. *Am J Pathol* 1991;139:461–466.
- [41] Foxall C, Watson SR, Dowbenko D, et al. The three members of the selectin receptor family recognize a common carbohydrate epitome, the sialyl Lewis^x oligosaccharide. *J Cell Biol* 1992;117:895–902.
- [42] Buerke M, Weyrich AS, Zheng Z, Gaeta FCA, Forrest MJ, Lefer AM. Sialyl Lewis^x-containing oligosaccharide attenuates myocardial reperfusion injury in cats. *J Clin Invest* 1994;93:1140–1148.
- [43] Lefer DJ, Flynn DM, Phillips ML, Ratcliffe M, Buda AJ. A novel sialyl Lewis^x analog attenuates neutrophil accumulation and myocardial necrosis after ischemia and reperfusion. *Circulation* 1994;90:2390–2401.
- [44] Murohara T, Margiotta J, Phillips LM, et al. Cardioprotection by liposome-conjugated sialyl Lewis^x-oligosaccharide in myocardial ischaemia and reperfusion injury. *Cardiovasc Res* 1995;30:965–974.
- [45] Skurk C, Buerke M, Guo J-P, Paulson J, Lefer AM. Sialyl Lewis^x-containing oligosaccharide exerts beneficial effects in murine traumatic shock. *Am J Physiol* 1994;267:H2124–H2131.
- [46] Granger DN, Kubes P. The microcirculation and inflammation: modulation of leukocyte–endothelial cell adhesion. *J Leukocyte Biol* 1994;55:662–675.
- [47] Davenpeck KL, Gauthier TW, Albertine KH, Lefer AM. Role of P-selectin in microvascular leukocyte–endothelial interaction in splanchnic ischemia–reperfusion. *Am J Physiol* 1994;267:H622–H630.
- [48] Ma X-L, Weyrich AS, Lefer DJ, Lefer AM. Diminished basal nitric oxide release after myocardial ischemia and reperfusion promotes neutrophil adherence to coronary endothelium. *Circ Res* 1993;72:403–412.
- [49] Johnson G III, Tsao PS, Lefer AM. Cardioprotective effects of authentic nitric oxide in myocardial ischemia with reperfusion. *Crit Care Med* 1991;19:244–252.
- [50] Aoki N, Johnson G III, Lefer AM. Beneficial effects of two forms of NO administration in feline splanchnic artery occlusion shock. *Am J Physiol* 1990;258:G275–G281.
- [51] Johnson G III, Tsao PS, Mulloy D, Lefer AM. Cardioprotective effects of acidified sodium nitrite in myocardial ischemia with reperfusion. *J Pharmacol Exp Ther* 1990;252:35–41.
- [52] Siegfried MR, Carey C, Ma X-L, Lefer AM. Beneficial effects of SPM-5185, a cysteine-containing NO donor in myocardial ischemia–reperfusion. *Am J Physiol* 1992;263: H771–H777.
- [53] Lefer DJ, Nakanishi K, Johnston WE, Vinten-Johansen J. Antineu-

- trophil and myocardial protecting actions of a novel nitric oxide donor after acute myocardial ischemia and reperfusion in dogs. *Circulation* 1993;88:2337–2350.
- [54] Siegfried MR, Erhardt J, Rider T, Ma X-L, Lefer AM. Cardioprotection and attenuation of endothelial dysfunction by organic nitric oxide donors in myocardial ischemia–reperfusion. *J Pharmacol Exp Ther* 1992;260:668–675.
- [55] Pabla R, Buda AJ, Flynn DM, Salzberg DB, Lefer DJ. Intracoronary nitric oxide improves postischemic coronary blood flow and myocardial contractile function. *Am J Physiol* 269:H1113–H1121.
- [56] Weyrich AS, Ma X-L, Lefer AM. The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. *Circulation* 1992;86:279–288.
- [57] Nakanishi K, Vinten-Johansen J, Lefer DJ, Zhao Z, Fowler WC III, McGee DS, Johnston WE. Intracoronary L-arginine during reperfusion improves endothelial function and reduces infarct size. *Am J Physiol* 1992;263:H1650–H1658.
- [58] Weyrich AS, Ma X-L, Buerke M, et al. Physiological concentrations of nitric oxide do not elicit an acute negative inotropic effect in unstimulated cardiac muscle. *Circ Res* 1994;75:692–700.
- [59] Radomski MW, Palmer RMJ, Moncada S. The anti-aggregating properties of vascular endothelium: Interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987;92:639–644.
- [60] Kubes P, Suzuki M, Granger D. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 1991;88:4651–4655.
- [61] Davenpeck KL, Gauthier TW, Lefer AM. Inhibition of endothelial-derived nitric oxide promotes P-selectin expression and actions in the rat microcirculation. *Gastroenterology* 1994;107:1050–1058.
- [62] Rubanyi GM, Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 1986;250:H822–H827.
- [63] Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* 1986;320:454–456.
- [64] McCall T, Whittle BJR, Boughton-Smith NK, Moncada S. Inhibition of fMLP induced aggregation of rabbit neutrophils by nitric oxide. *Br J Pharmacol* 1988;85:517P.
- [65] Carey C, Siegfried MR, Ma X-L, Weyrich AS, Lefer AM. Anti-shock and endothelial protective actions of a NO donor in mesenteric ischemia and reperfusion. *Circ Shock* 1992;38:209–216.
- [66] Clancy RM, Leszczynska-Piziak J, Abramson SB. Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action in the NADPH oxidase. *J Clin Invest* 1992;90:1116–1121.
- [67] Moilanen E, Vuorinen P, Kankaanranta H, Metsä-Ketela T, Vapatalo H. Inhibition by nitric oxide-donors of human polymorphonuclear leukocyte function. *Br J Pharmacol* 1993;109:852–858.
- [68] Forslund T, Sundqvist T. Nitric oxide reduces hydrogen peroxide production from human polymorphonuclear neutrophils. *Eur J Clin Invest* 1995;25:9–14.
- [69] Lefer DJ, Klunk DA, Luty GA, Merges C, Schleimer RP, Bochner BS, Zweier JL. Nitric oxide (NO) donors reduce basal ICAM-1 expression on human aortic endothelial cells (HAEC's). *Circulation* 1993;88[part 2]:I-565 (abstract).
- [70] Gauthier TW, Davenpeck KL, Lefer AM. Nitric oxide attenuates leukocyte–endothelial interaction via P-selectin in splanchnic ischemia–reperfusion. *Am J Physiol* 1994;267:G562–G568.