Heparinase III Exerts Endothelial and Cardioprotective Effects in Feline Myocardial Ischemia-Reperfusion Injury

REID HAYWARD, TARECK O. NOSSULI and ALLAN M. LEFER
Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania
Accepted for publication August 15, 1997

ABSTRACT
The initial phase of neutrophil (PMN) adherence in the pathophysiology of myocardial ischemia-reperfusion (MI/R) injury depends on the selectins, particularly P- and L-selectin. Several ligands for these selectins have been identified, one of which may be a heparan sulfate proteoglycan (HSPG). Cats subjected to 90 min of MI and 270 min of R were given either heparinase III (0.033, 0.33 or 3.33 IU/kg/min) or its vehicle beginning 10 min before R and continuing throughout the 270-min R period. Heparinase III at 3.33 IU/kg/min provided a marked cardioprotective effect compared with cats receiving only vehicle as evidenced by a significant attenuation in myocardial necrosis (P < .01). In addition, endothelium-dependent vasorelaxation to acetylcholine in coronary artery rings isolated from MI/R cats treated with heparinase III was significantly preserved (P < .01). Adherence of PMNs to the coronary vascular endothelium after 270 min of R was also significantly attenuated in heparinase III-treated cats compared with vehicle (P < .01). At 0.33 IU/kg/min, heparinase III exerted modest, significant cardioprotective effects, whereas at 0.033 IU/kg/min, no significant beneficial effects were observed. Our results indicate that heparinase III is cardioprotective in a dose-dependent manner, preserves endothelial function and attenuates PMN adherence to the coronary vascular endothelium.

The selectins are a family of three cell surface glycoproteins sharing a high degree of functional and structural characteristics. These glycoprotein adhesion molecules are responsible for much of the initial interaction occurring between leukocytes and endothelial cells during the progression of acute inflammation. The interaction of leukocytes with endothelial cells begins with PMN rolling, which is attributed to the rapid expression of P-selectin on the endothelial surface and constitutively expressed L-selectin on PMNs (Lawrence and Springer, 1991; McEver, 1991). Studies demonstrating the role of these selectins in the pathophysiology of MI/R have shown that monoclonal antibodies directed against either P- or L-selectin substantially attenuate PMN adherence to vascular endothelium thereby decreasing myocardial necrosis and preserving coronary endothelial function (Ma et al., 1993; Weyrich et al., 1993).

Several studies investigating the carbohydrate ligands of the selectins have focused primarily on sialylated fucosylated structures such as sLeα (Buerke et al., 1994b). However, recent attention has been focused on structures unrelated to sLeα. Soluble P- and L-selectin have been shown to bind both heparin and heparin-like molecules (Bevilacqua and Nelson, 1993; Norgard-Sumnicht et al., 1993). Nelson et al. (1993) demonstrated that exogenous heparin and heparan sulfate

ABBREVIATIONS: PMN, polymorphonuclear leukocyte or neutrophil; MI/R, myocardial ischemia-reperfusion; HSPG, heparan sulfate proteoglycan; HS, heparan sulfate; PBS, phosphate buffered saline; sLeα, sialyl Lewisα; MABP, mean arterial blood pressure; LAD, left anterior descending; ECG, electrocardiogram; HR, heart rate; PRR, pressure-rate index; K-H, Krebs-Henseleit; LCX, left circumflex; NBT, nitroblue tetrazolium; PPP, platelet-poor plasma; ACh, acetylcholine.
inhibit the binding of P- and L-selectin to sLe\(^{\alpha}\) as well as to a related structure, sialyl Lewis\(^{a}\). Several studies point to a HSPG whose glucosamine residues are enriched with unsubstituted amino groups (Norgard-Sumnicht and Varki, 1993). Although there are several common structural characteristics among the selectins, E-selectin does not appear to demonstrate heparin oligosaccharide-binding properties (Nelson et al., 1993). Moreover, E-selectin is not a key cell adhesion molecule in MI/R (Weyrich et al., 1995).

In addition to its role as a selectin ligand, endothelial cell HSPG have been suggested to play a key role in the presentation of chemokines to circulating leukocytes. Chemokines are a group of heparin-binding cytokines that are produced by several cells upon activation (Baggiolini et al., 1994). These chemokines modulate leukocyte recruitment and extravasation by chemotaxis and by stimulating integrin-ligand interactions. Heparinases are a class of enzymes in the development and progression of leukocyte-endothelial cell interactions. Heparinases are capable of degrading heparin, heparinase II primarily cleaves heparin, heparinase II capable of degrading heparin as well as heparan sulfate. E-selectin is not a key cell adhesion molecule in MI/R (Weyrich et al., 1995). Moreover, E-selectin is not a key cell adhesion molecule in MI/R (Weyrich et al., 1995).

Several studies have confirmed that endothelial cells synthesize heparan sulfate (Castillo et al., 1987; Kinsella and Wight, 1988) which is subsequently incorporated into a more complex molecule, HSPG. In addition, the surface expression and modification of surface expressed HSPG appears to be highly regulated and can be influenced by several factors including neutrophils (Key et al., 1992), lymphocytes (Geller et al., 1994), cytokines and endotoxin (Klein et al., 1992). Recently, attention has focused on the function of heparinases in the development and progression of leukocyte-endothelial cell interactions. Heparinases are a class of enzymes capable of degrading heparin as well as heparan sulfate. Heparinase I primarily cleaves heparin, heparinase II cleaves both heparin and heparan sulfate, and heparinase III selectively cleaves heparan sulfate (Lohse and Linhardt, 1992; Yang et al., 1985). Moreover, heparinase III has been shown to inhibit neutrophil extravasation in vitro (Bennett et al., 1997). Therefore, the purpose of this investigation was to determine the effects of varying doses of heparinase III on myocardial tissue injury, coronary endothelial function and adherence of PMNs to the coronary vascular endothelium in a well established model of feline MI/R.

**Methods**

**MI/R in vivo.** Adult male cats (2.6–3.6 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.). An intratracheal cannula was inserted through a midline incision, and cats were placed on mechanical ventilation (Harvard small animal respirator, Dover, MA). A catheter was inserted into the jugular vein for administration of additional anesthesia and for administration of heparinase III, or an equivalent volume of vehicle (PBS). Another catheter was inserted into the right femoral artery and used in the measurement of MABP via a pressure transducer (Cobe Instruments, Lakewood, CO). After a midsternal thoracotomy, the anterior pericardium was incised and a 3–0 silk ligature was placed around the LAD coronary artery 8 to 10 mm from its origin. Standard lead II of the scalar ECG was used to determine HR and S-T segment elevation. The ECG and MABP were continuously monitored on a model 78304 A unit oscilloscope (Hewlett Packard, Palo Alto, CA) and recorded on an oscillographic recorder (Gould, model 2107–4490-00, Cleveland, OH) every 20 min. The PRI, an index of myocardial oxygen demand, was calculated as (MABP × HR)/1000.

**Experimental protocol.** After completion of all surgical procedures, the cats were allowed to stabilize for 30 min before baseline readings of ECG and MABP were recorded. In cats subjected to MI/R, ischemia was induced by tightening the previously placed reversible silk ligature around the LAD so that the vessel was completely occluded. This was designated as time 0. Eighty minutes after coronary occlusion (i.e., 10 min before R) an infusion of heparinase III or its vehicle was initiated, and continued for the duration of the R period. After the 90-min ischemic period, the LAD ligature was untied and the ischemic myocardium was allowed to reperfuse for 270 min.

Twenty-eight cats were randomly divided into several major groups consisting of five to six cats/group: 1) sham MI/R cats receiving heparinase III (3.33 IU/kg/min); 2) MI/R cats receiving PBS alone as a vehicle; and 3) MI/R cats receiving heparinase III (0.033, 0.33 or 3.33 IU/kg/min). Sham MI/R cats were subjected to the same surgical procedures and observed for the same duration of time as MI/R cats except that the LAD coronary artery was not occluded.

The gene for heparinase III was isolated from Flavobacterium heparinum and produced from Escherichia coli as described previously (Su et al., 1996). Heparinase III (49 IU/mg protein) was manufactured and provided by IBEX Technologies, Inc., Montréal, Québec, Canada.

**Quantification of myocardial area-at-risk and necrotic area.** At the end of the 270-min reperfusion, the ligature around the LAD was again tightened. Twenty milliliters of 0.5% Evans blue (Sigma Chemical Co., St. Louis, MO) was injected directly into the left ventricle (Sigma Chemical Co., St. Louis, MO) was injected directly into the left ventricle via the left atrium using a 19-gauge needle. The heart was then rapidly excised and placed in warmed, oxygenated K-H buffer consisting of (in mM): NaCl, 118; KCl, 4.75; CaCl\(_{2}\), 1.19; MgSO\(_4\)-7H\(_2\)O, 1.19; NaHCO\(_3\), 12.5; and glucose 10.0, pH 7.4. The LCX and LAD coronary arteries were isolated for subsequent study of coronary ring vasoactivity and PMN adherence. The right ventricle, great vessels and fat tissue were removed, and the area-at-risk which did not stain was separated from the stained portion of the myocardium. The irreversibly injured or necrotic portion of the myocardium at risk which did not stain was separated from the stained portion of the myocardium. The gene for heparinase III was isolated from Flavobacterium heparinum and produced from Escherichia coli as described previously (Su et al., 1996). Heparinase III (49 IU/mg protein) was manufactured and provided by IBEX Technologies, Inc., Montréal, Québec, Canada.

**Autologous cat PMN isolation and labeling.** Peripheral blood (20 ml) was collected from the femoral artery at the beginning of the surgical procedure and anticoagulated with citrate-phosphate-dextrose solution (Sigma) (1.5:10, vol/vol anticoagulant to whole blood). PMNs were isolated by a procedure modified from Lafrado and Olsen (1986). After whole blood centrifugation, the pellet was mixed with 8 ml of 6% dextran (MW 60,000–90,000; Sigma) and PBS to allow the red blood cells to settle. The leukocyte-rich upper fraction was layered onto a Percoll/PPP gradient (density gradients of 80%, 62% and 50%). After centrifugation at 3000 rpm for 40 min, PMNs were collected from the 62% and 80% interface and washed in PBS. PMN preparations obtained by this method were generally >95% pure and...
95% viable. Isolated PMNs were then labeled with a Zynaxis PKH-2 cell linker (Zynaxis Cell Science Inc., prepared for Sigma Immunochemicals, Malvern, PA) based on the procedure of Yuan and Fleming (1990). Two milliliters of diluent and 10 μl dye were added to a loose cell pellet containing approximately 10 million cells. After a 7-min incubation period, 200 ml of PPP were added to stop the reaction and 2 ml of PBS were added to underlay the suspension. The mixture was then centrifuged for 10 min at 1800 rpm. The cells were resuspended in PBS, counted and used in the adherence assay.

**PMN adherence to the ischemic-reperfusion coronary endothelium.** PMNs were isolated and fluorescently labeled as described above. Segments from both the LAD and LCX coronary arteries were isolated from each cat and placed into warmed K-H buffer. Arteries were cut into rings of 2 to 3 mm length. The rings were then opened and placed with the endothelial surface up into a cell culture dish filled with 3 ml of oxygenated K-H solution and incubated in culture dishes with autologous labeled PMNs (1.2 x 10⁶ cells) for 20 min at 37°C. After the 20-min incubation period, sections were washed in K-H buffer and placed on glass microscope slides. PMNs adhering to the endothelium were counted by epifluorescence microscopy (Nikon Diaphot, Nikon Inc., Garden City, NY). Five different fields of each endothelial surface were counted and the results expressed as adherent PMNs/mm² of endothelial surface.

**Isolated coronary artery ring vasoactivity.** The LAD and LCX coronary arteries were isolated and placed into warmed K-H solution as described above. Arteries were cut into rings of 2 to 3 mm length. The rings were then mounted on stainless steel hooks, transferred to tissue baths and connected to PT-03 force transducers (Grass, model 7, Instrument Co., Quincy, MA). Isometric force changes were recorded on a Grass-7 oscillographic recorder. The baths were filled with 10 ml of K-H solution and gassed with 95% O₂/5% CO₂ at 37°C. After the 20-min incubation period, sections were washed in K-H buffer and placed on glass microscope slides. PMNs adhering to the endothelium were counted by epifluorescence microscopy (Nikon Diaphot, Nikon Inc., Garden City, NY). Five different fields of each endothelial surface were counted and the results expressed as adherent PMNs/mm² of endothelial surface.

**Results**

**Electrophysiological, hemodynamic and white blood cell parameters.** Before coronary artery occlusion, all cats demonstrated comparable values in all of the observed variables. Immediately after occlusion of the LAD coronary artery, the portion of the left ventricle that had been perfused by the occluded artery became cyanotic and was accompanied by substantial elevation in the S-T segment. At the end of the 90-min ischemic period, the ligature around the LAD was untied. Reperfusion was confirmed by the return of the ischemic region of the myocardium to normal color and a marked decline in the S-T segment elevation of the ECG. All MI/R groups demonstrated substantial S-T segment elevation, with peak values occurring between 20 and 40 min after coronary occlusion (fig. 1). There were no significant differences in peak S-T segment elevation among the MI/R groups, which suggested that the degree of ischemia was similar among all ischemic groups. Similarly, in all groups of MI/R cats, the PRI decreased significantly after coronary occlusion and slowly increased after reperfusion (fig. 2). No significant differences in PRI were observed among any of the MI/R groups throughout the entire data collection period. Circulating white blood cell counts were determined immediately before coronary occlusion and at 1, 2, 4 and 6 h after coronary occlusion. All groups of cats demonstrated the same time course of circulating leukocyte changes, exhibiting an initial decline and a subsequent return to control values by the end of the reperfusion period (fig. 3). No significant differences were observed among any of the groups regarding circulating leukocyte counts. These results clearly indicate that any observed cardioprotective effects of heparinase III cannot be attributed to alterations in either myocardial oxygen demand, or in differences in the number of circulating white blood cells available to the reperfused myocardium.

**Effects of heparinase III on myocardial injury.** To determine the ability of heparinase III to protect the myocardium after MI/R, we measured the amount of necrotic tissue relative to the area-at-risk (fig. 4). There were no significant differences in the area-at-risk among any of the MI/R groups, confirming that a similar mass of myocardium was jeopardized in all groups. However, comparison of the necrotic areas indexed to the area-at-risk shows that administration of heparinase III at 0.33 or 3.33 IU/kg/min resulted in significant dose-dependent reductions in myocardial necrosis compared with MI/R cats receiving only vehicle (i.e., PBS). The area-of-necrosis to area-at-risk was 27 ± 3% in cats receiving only PBS, whereas the area-of-necrosis to area-at-risk in the heparinase III-treated animals was reduced by 48% (P < .01), 33% (P < .05) and 9% (NS) in the 3.33, 0.33 and 0.033 IU/kg/min groups, respectively. Similar results were observed when the area of necrosis was indexed to the total left ventricular mass (fig. 4).

**Effects of heparinase III on endothelial dysfunction.** Figure 5 summarizes the vasorelaxant responses of isolated cat coronary artery rings to ACh and acidified NaNO₂. Cor-
Coronary artery rings isolated from sham-operated control cats demonstrated nearly total relaxation to all vasodilators studied. However, the response of LAD coronary artery rings (i.e., ischemic-reperfused) to the endothelial-dependent vasodilator ACh were significantly (P < .01) attenuated in cats receiving only the vehicle. However, heparinase III treatment significantly preserved LAD endothelial function at both the 3.33 IU/kg/min as well as the 0.33 IU/kg/min dosage (P < .01 from untreated MI/R). A significant loss of vasodilator responsiveness was observed at the 0.033 IU/kg/min dose (P < .05), which indicates a substantial degree of endothelial dysfunction. Control LCX coronary artery rings maintained both endothelial-dependent as well as endothelial-independent vasorelaxation in all groups.

The direct vascular effects of heparinase III were also examined in both LAD and LCX coronary artery rings in vitro because a direct vasodilator effect could be cardioprotective in the ischemic-reperfused heart. Heparinase III was administered to isolated LAD and LCX coronary artery rings at concentrations of 0.066 and 0.666 IU/ml. These concentrations were selected because they approximate in vivo circulating concentrations based on estimated blood volume and a dose of 3.33 IU/kg. Heparinase III treatment did not significantly alter basal vascular tone after precontraction with U-46619. Coronary artery rings relaxed 0.56 ± 0.1% after addition of 0.066 IU/ml and 0.8 ± 0.3% after addition of 0.666 IU/ml heparinase III to the bath. These values (<1% vasorelaxation) are trivial in comparison with a relaxation of 83 ± 4% to ACh in the same coronary artery rings, and indicate that heparinase III does not induce coronary vasoactivity at concentrations expected after infusion of cardioprotective doses.

Effect of heparinase III on PMN adherence to ischemia-reperfused coronary endothelium. An initial step in the PMN-mediated reperfusion injury is the increased...
adhesiveness of the vascular endothelium. Thus, we measured the ex vivo adherence of PMNs after the in vivo administration of heparinase III (fig. 6). When unstimulated autologous PMNs were incubated with nonischemic LCX coronary artery segments for 20 min, relatively few became adherent to the endothelium regardless of group (<40/mm²). However, in MI/R cats receiving only PBS, unstimulated PMNs incubated with MI/R LAD coronary artery segments resulted in a significant increase in adherence (P < .01). When autologous unstimulated PMNs were incubated with LAD segments isolated from cats treated with heparinase III, there was a significant attenuation in adherence; this effect occurred in a dose-dependent fashion. Pretreatment with 3.33 IU/kg/min heparinase III resulted in a 60% (P < .01) reduction in adherent PMNs to the LAD endothelium, whereas pretreatment with 0.33 IU/kg/min heparinase III reduced adherence by 33% (P < .05). Cats pretreated with 0.033 IU/kg/min heparinase III demonstrated no significant differences from MI/R cats receiving only PBS.

Discussion

Our results clearly demonstrate a cardioprotective effect of in vivo administration of heparinase III in our feline model of MI/R. Despite the fact that all MI/R groups were subjected to a similar degree of myocardial jeopardy (i.e., similar S-T segment elevations and areas-at-risk), heparinase III at 3.33 IU/kg/min reduced myocardial necrosis by approximately 50% in comparison with cats receiving only its vehicle. Because cats possess minimal collateral circulation (Greve et al., 1989) and heparinase III treatment did not affect the PRI or coronary vascular tone in vitro, it appears as though the cardioprotective effects are not caused by overt alterations in myocardial oxygen demand or supply. Depletion of circulating leukocytes provides cardioprotective effects after MI/R because of a reduction of PMN accumulation in the reperfused myocardium (Romson et al., 1983). Our results do not support the possibility of differences in circulating leukocytes as a factor in the protective mechanism of heparinase III as evidenced by similar white blood cell counts at all times for all groups of cats.

Although a few studies have been conducted with heparinase III in the setting of acute inflammation, our data are consistent with those results. Bennett et al. (1997) reported that in vitro heparinase III treatment decreased L-selectin binding by 50% and inhibited neutrophil extravasation up to 30%. In a rabbit model of MI/R, myocardial necrosis was reduced by 40% in animals undergoing heparinase III treatment in comparison to animals receiving only vehicle (Kingma et al., 1997).

The contribution of neutrophils to the development and progression of myocardial injury after ischemia and reperfusion has been clearly established (Entman and Smith, 1994; Lefer and Lefer, 1993; Lucchesi et al., 1989). Neutrophil extravasation from the vascular lumen to the site of inflammation is a three-step process (Butcher, 1991). Initially, PMNs within the vascular lumen must recognize and interact with activated vascular endothelium, which results in a characteristic rolling action caused by the balance of adhesive forces and vascular shear forces. This is followed by a strengthening of adhesive forces which results in the capture and firm adherence of PMNs to the endothelium. Finally, many of the adherent PMNs extravasate through the endothelium and come in close proximity to cardiac myocytes where they mediate their injurious effects. Some of the most successful therapeutic techniques are targeted at the initial step of PMN-endothelial cell interactions, effectively inhibiting leukocyte rolling (Davenpeck et al., 1994) and thereby preventing PMNs from extravasating across the endothelium and reaching the target tissue (Buerke et al., 1994a; Ma et al., 1993; Murohara et al., 1996).

Attention has focused on the molecular basis of selectin adhesion, much of which has centered around the ability of the lectin domain to recognize carbohydrate structures. The identification of selectin ligands is difficult because each of the selectins can bind to several carbohydrate structures. However, each selectin does not possess equivalent binding affinity to these carbohydrate structures. For example, E-selectin has a high affinity for the tetrasaccharide sLeα whereas L-selectin has a low affinity for sLeα and a high affinity for sulflo-Leα-derived molecules (Green et al., 1995). E-selectin also appears to bind with high affinity to more complex carbohydrate structures such as N-linked tetra-antennary difucosyl oligosaccharides (Patel et al., 1994) as well as noncarbohydrate phosphorylated or polysulfated molecules such as inositol polyynolans (Cocconi et al., 1994). Additionally, P- and L-selectin, but not E-selectin have been shown to bind both heparin and heparin oligosaccharide structures (Green et al., 1995; Nelson et al., 1993). Thus, there are clearly several ligands which bind to each of the selectins. Recently, PSGL-1 has been identified as a major high-affinity ligand for P-selectin (McEver et al., 1995).

Recent evidence regarding the role of HSPGs as ligands for specific selectin adhesion molecules suggests that the cardioprotective effects of heparinase III may reside in its ability to inhibit the initial PMN-endothelial cell interactions (i.e., rolling) primarily via L-selectin-mediated action. L-selectin, in contrast to P- and E-selectins, which are located on endothelium, is constitutively expressed on the surface of neutrophils, lymphocytes and monocytes. L-Selectin is believed to mediate the first step of neutrophil contact with the endothelium (Whelan, 1996). Thus, the potential importance of inhibiting L-selectin-mediated leukocyte-endothelial cell inter-
actions lies in its ability to limit the progression of neutrophils from the vascular lumen to the target tissue, thereby reducing PMN-mediated tissue destruction.

Because HSPGs have been expressed on the luminal surface of endothelial cells (Cavari and Vannuchi, 1993; De Agostini et al., 1990), these ligands are believed to contribute to early leukocyte-endothelial cell interactions. However, the L-selectin-binding HSPG sequences have been suggested to be localized in the extracellular basement membrane, and if so, their ability to bind with L-selectin may be significant only when the junctions between endothelial cells are separated (e.g., when the endothelium is injured or inflamed) (Norgard-Sumnicht and Varki, 1995). The cardioprotective actions of heparinase III may be closely associated with an inhibition of leukocyte-endothelial cell interactions. Cell surface HSPG serves as a ligand for several molecules including extracellular enzymes such as lipoprotein lipase (Cheng et al., 1981) and superoxide dismutase (Adachi and Marklund, 1989). Because superoxide dismutase provides cardioprotection in this model of ischemia-reperfusion (Tsao et al., 1990), the degradation of its endothelial cell ligand may diminish what may serve as a first line of defense against oxygen-derived free radicals released by activated neutrophils.

An important mechanism of action associated with the administration of heparinase III in the feline model of MI/R may involve inhibition of selectin-mediated leukocyte-endothelial cell interactions. Up-regulation of P-selectin on the vascular endothelium is observed after 20 min of reperfusion (Weyrich et al., 1995) whereas L-selectin is constitutively expressed on PMNs that bind to the ligands on the reperfused endothelium (Von Andrian et al., 1991). In contrast, E-selectin is not up-regulated on the endothelial surface until approximately 4 to 6 h post-reperfusion (Bevilacqua and Nelson, 1993; Weyrich et al., 1995). Thus, E-selectin does not bind heparin oligosaccharide structures, and this selectin would be only minimally involved in our model because it is not up-regulated during the time when myocardial necrosis is developing.

The degree of cardioprotection associated with heparinase III administration is in agreement with other studies investigating the role of L-selectin in MI/R. Monoclonal antibodies directed against L-selectin have been shown to block up to 80% of intravascular neutrophil rolling and subsequently reducing adherence in mesenteric venules (Smith et al., 1991; Spertini et al., 1991). Such antibodies have also reduced myocardial necrosis by 50 to 60% after MI/R in cats (Buerke et al., 1994b; Ma et al., 1993). The use of soluble SLeα oligosaccharides appears to result in even greater myocardial protection with necrosis attenuated by almost 80% in the feline MI/R model (Buerke et al., 1994c). The failure of monoclonal antibodies, sialylated oligosaccharides or heparinase III alone to completely eliminate leukocyte-endothelial cell interactions or myocardial necrosis suggests that multiple carbohydrate ligands are functioning in vivo. In addition, these results may indicate a substantial contribution of HSPGs as an endothelial ligand for L-selectin because similar results were obtained when L-selectin was specifically targeted (i.e., monoclonal antibodies or SLeα oligosaccharide) or when one of the identified ligands for L-selectin is targeted (i.e., heparinase III). Thus, heparinase III may be considered as a potentially useful candidate substance to be given adjunc-tively with a thrombolytic agent at the time of thrombolysis to limit reperfusion injury.

Acknowledgments

The authors gratefully acknowledge Robert Craig (TJU) for his excellent technical assistance in the biochemical analysis and Dr. Paul Silver (IBEX Technologies, Inc.) for his aid in designing these studies and for his critical review of the manuscript.

References


Downloaded from jpet.aspetjournals.org at ASPET Journals on November 12, 2017


Send reprint requests to: Allan M. Lefer, Ph.D., Department of Physiology, Jefferson Medical College, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107-6799.