

Effect of Sialyl Lewis^x Selectin Blockade on Myocardial Protection During Cardioplegic Arrest and Reperfusion

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ABSTRACT

Purpose: Selectins play a crucial role in the neutrophil-mediated myocardial injury associated with ischemia/reperfusion. We investigated the effect of selectin inhibition on neutrophil-endothelial cell adhesion, myocardial water content, and left ventricular (LV) recovery after cardiopulmonary bypass (CPB) and cardioplegia.

Methods: Dogs were subjected to CPB and 60 minutes of hypothermic cardioplegia. A selectin inhibitor (SI) (25 mg/kg) was given five minutes prior to CPB and as a continuous infusion (5 mg/kg/h) throughout CPB (n = 6). Saline-treated controls (n = 6) received identical volumes. Preload recruitable stroke work (PRSW) was calculated by sonomicrometry and micromanometry. Myocardial water content was determined by microgravimetry. Myeloperoxidase (MPO) activity was measured to quantify polymorphonuclear neutrophil (PMN) infiltration.

Results: SI did not attenuate PRSW as well as post-CPB MPO tissue activity. While we found no difference in myocardial water gain between groups 120 minutes post-CPB, there was better edema resolution with SI.

Conclusions: Selectin antagonism does not reduce CPB-associated myocardial injury, and contractile recovery is not enhanced.

INTRODUCTION

Myocardial protection by means of cardioplegic arrest offers enhanced tissue protection and provides the surgeon with a controlled environment in which to perform precision heart surgery. Despite these benefits, the sequelae of cardioplegia and cardiopulmonary bypass (CPB) may be detrimental. This study focuses attention on one aspect of myocardial injury that is known to be accelerated by reperfusion [Braunwald 1985, Hearse 1992], the accumulation of polymorphonuclear neutrophils (PMN). Its pathogenesis is multifactorial but is believed to be triggered by a cascade of inflammatory events characterized by the elaboration of cytokines

and proinflammatory mediators, which in turn lead to the expression of cell adhesion molecules and chemoattraction of PMNs. Subsequently PMNs become adherent to the microvasculature and migrate into the interstitium. The release of PMN-mediated products leads to microvascular barrier damage and interstitial edema, ultimately causing organ dysfunction [Dauber 1990, Dreyer 1991]. In addition, sequestration of neutrophils can result in mechanical plugging of capillaries, described as the "no-reflow" phenomenon [Kloner 1974, Argano 1996].

Although there may be continuing controversy [Kurose 1994, Gill 1996], numerous studies provide evidence supporting the importance of neutrophils in myocardial injury associated with CPB [Wilson 1993, Schmidt 1996, Tofukuji 1998] and suggest that PMNs are partly responsible for the myocardial dysfunction that follows ischemia [Bolli 1992]. It is conceivable, therefore, that by inhibiting one of the factors that promote leukocyte-endothelial cell interactions it may be possible to attenuate the myocardial injury following CPB.

The selectin family of adhesion molecules plays a pivotal role in the neutrophil-mediated injury [Lefer 1994], and recent studies indicate that administration of soluble selectin ligands may have the potential to attenuate endothelial interactions [Foxall 1992, Flynn 1996, Palma-Vargas 1997, Yamada 1998]. As neutrophil activation is a prominent feature of myocardial injury, we postulated that the inhibition of selectin binding proteins with a novel small molecule, non-oligosaccharide sialyl Lewis^x (sLe^x) analogue of P-, E-, and L-selectin binding [Kogan 1998], would attenuate the degree of myocardial injury encountered during cardiac surgery and reduce PMN sequestration by decreasing PMN adhesion to endothelial cells. To test this hypothesis, we subjected dogs to CPB and cardioplegic arrest followed by reperfusion in the presence or in the absence of the selectin inhibitor. Specifically, we sought (1) to examine the ability of the sLe^x inhibitor to reduce the increase in myocardial water content, (2) to assess its effect on the myocardial sequestration of PMN during reperfusion, and (3) to investigate whether a sLe^x analogue improves the recovery of post-cardioplegia myocardial contractility.

MATERIALS AND METHODS

Animal Preparation

All procedures were approved by the University of Texas Animal Welfare Committee and were consistent with the

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National Institutes of Health “Guide for the Care and Use of Laboratory Animals” (NIH publication 85-23, revised 1985). Twelve conditioned mongrel dogs of either sex (34.2 ± 1.0 kg) were anesthetized by intravenous administration of 25 mg/kg thiopental sodium (Pentotal; Abbott Laboratories, North Chicago, IL), endotracheally intubated, and mechanically ventilated with 100% oxygen using a volume-cycled respirator (Siemens-Elema AB, Syndbyberg, Sweden). Anesthesia was maintained with intravenous infusion of 1% thiopental sodium in Ringer’s solution.

Fluid-filled catheters were placed into the left femoral artery and vein for arterial pressure monitoring, arterial blood sampling, and fluid administration, respectively. A 7F Swan-Ganz thermodilution catheter was inserted into the pulmonary artery via the right jugular vein for pressure and cardiac output measurements. We then exposed the right femoral artery for subsequent CPB cannulation. After a median sternotomy and pericardiotomy, a 5F catheter was advanced into the coronary sinus via a snare-secured purse stitch for coronary sinus blood sampling. An umbilical tape was placed around the inferior vena cava for cardiac preload manipulation. A micromanometer-tipped pressure transducer (Millar Instruments Inc., Houston, TX) was introduced into the left ventricle cavity through the apex. Sonomicrometry crystals (10 MHz) (Sonometrics, London, ON, Canada) were placed in the left ventricular (LV) subendocardium across the septum/free-wall axis of the LV. The crystals were then connected to a sonomicrometer (Triton Technology Inc.) for processing of signals.

Hemodynamics and Preload Recrutable Stroke Work

Hemodynamic data were simultaneously logged into a Macintosh Quadra 700 computer via an analog-to-digital data acquisition device (MacLab, World Precision Instruments Inc., New Haven, CT). Cardiac output was determined in duplicate by injecting 10 mL ice-cold Ringer’s solution. LV pressure was measured with a micromanometer and the left ventricular septum/free-wall diameter was obtained with a sonomicrometer. These data were recorded at a frequency of 200 Hz during 10 seconds of inferior vena cava occlusion (Sonolab/Sonoview[®], Sonometrics, London, ON, Canada). If we assume a spherical shape, the LV volume (V_{LV}) can be calculated from the following equation:

$$V_{LV} = (4/3) \cdot \pi \cdot (r_{LV})^3 \quad [\text{mL}]$$

where r_{LV} is the radius of the LV. Replacing r_{LV} with $(10^{-1} \cdot d_{LV}/2)$ yields

$$V_{LV} = \pi \cdot (d_{LV})^3 / 6000 \quad [\text{mL}]$$

Preload recruitable stroke work (PRSW) was calculated as the slope of the relation between LV end-diastolic volume and LV stroke work (SW). SW was calculated as

$$SW = SV \cdot (\text{MEP} - \text{EDP}) \quad [\text{mL} \cdot \text{mm Hg}]$$

where SV is the LV stroke volume and MEP is the LV mean ejection pressure, measured from ejection onset to end ejection.

Ejection onset was defined at 10 ms after the time of $+dP/dt_{\text{max}}$, and end ejection was defined at the time of $-dP/dt_{\text{max}}$. EDP is the LV end-diastolic pressure. All measurements were taken in duplicate.

Myocardial Fluid Balance Parameters

Myocardial water content for edema quantification was measured using a microgravimetric technique previously described [Mehlhorn 1995]. With a biopsy needle (Tru-Cut[®], Baxter Healthcare Corp., Deerfield, IL), transmyocardial biopsies were taken from the LV anterior or anterolateral wall. The specific density of these samples was measured in a linear density gradient consisting of bromobenzene and kerosene. Knowing myocardial specific density, the gram water per gram tissue or myocardial water content (MWC) can be calculated using the following equation:

$$\text{MWC} = \{1 - [(SG_{\text{myo}}^{-1}) / (1 - 1/SG_{\text{dry}}) \cdot SG_{\text{myo}}]\} \cdot 100$$

where SG_{myo} and SG_{dry} are the specific gravities of the myocardial sample and of dry myocardium, respectively. All measurements were performed in triplicate and taken at baseline, 15 minutes after aortic cross-clamp removal, and at 10, 60, and 120 minutes post-CPB. The dogs were then killed and the hearts rapidly excised, after which both ventricles were weighed and dried to a constant weight at 60°C. We calculated SG_{dry} using the equation:

$$SG_{\text{dry}} = 1 / \{1 - [(SG_{\text{myo}} - 1) \cdot W / (D \cdot SG_{\text{myo}})]\}$$

where W and D are wet and dry weights of both ventricles. We assumed that SG_{dry} did not change over time.

Quantitative Assessment of PMN Infiltration

PMN infiltration was determined by measuring myeloperoxidase (MPO) tissue activity. MPO activity serves as a useful quantitative indicator for PMN accumulation because changes in activity correlate with histologic analyses of cell invasion [Mullane 1985]. To determine myeloperoxidase activity, we subjected myocardial tissue samples (500 mg each) to a spectrophotometric assay using the method of Mullane [Mullane 1985]. Briefly, LV samples were excised immediately at the end of the experiment and stored at -70°C until processed. Samples were pulverized after snap freezing with liquid nitrogen. Samples were then homogenized (10% wt/vol) with a Polytron homogenizer (Brinkman Model PT2000) in 50 mmol phosphate buffer solution (pH = 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide (Sigma) for 90 seconds. Homogenates were centrifuged at 12,500 rpm for 45 minutes (4°C). The supernatant was added to 0.166 mg/mL o-dianisidine dihydrochloride (Sigma), and 0.0005% hydrogen peroxide in 50 nm phosphate buffer (pH = 6.0). The change in absorbance was measured spectrophotometrically at 460 nm (Beckmann DU[®] 640) every 5 seconds for 2 minutes. Results were expressed as units of MPO/100 mg tissue (wet weight), where one unit of MPO activity was defined as the quantity of enzyme degrading 1 mmol peroxide/min at 25°C. The average for the duplicates was used for analysis.

Table 1. Hemodynamics and Left Ventricular Function in Test and Control Groups

A. The Selectin-Blockade Group							
Parameter	Baseline	Reperfusion		Post-CPB			
		1 min	15 min	10 min	30 min	60 min	120 min
CO (L·min ⁻¹)	2.57 ± 0.34	-	-	2.01 ± 0.28	1.90 ± 0.21	1.71 ± 0.25#	1.67 ± 0.29#
MAP (mm Hg)	104 ± 9	71 ± 3#	68 ± 3#	88 ± 2	85 ± 5#	92 ± 8	90 ± 10#
PAP (mm Hg)	16 ± 2	-	-	19 ± 2	18 ± 1	19 ± 1	19 ± 1
CVP (mm Hg)	6 ± 0	-	-	7 ± 1	7 ± 1	7 ± 0	7 ± 0#
PCWP (mm Hg)	9 ± 1	-	-	10 ± 1	10 ± 1	10 ± 0	9 ± 0
SVRI (dyn·s·m·cm ⁻²)	3307 ± 281	-	-	3397 ± 429	3554 ± 370	4397 ± 649	4215 ± 469
PVRI (dyn·s·m·cm ⁻²)	249 ± 40	-	-	357 ± 79	356 ± 46#	496 ± 86#	516 ± 99
B. The Control group							
Parameter	Baseline	Reperfusion		Post-CPB			
		1 min	15 min	10 min	30 min	60 min	120 min
CO (L·min ⁻¹)	2.21 ± 0.16	-	-	1.91 ± 0.18	2.18 ± 0.31	1.78 ± 0.25	1.71 ± 0.30
MAP (mm Hg)	113 ± 4	70 ± 2#	69 ± 3#	82 ± 3	102 ± 7	92 ± 8	98 ± 6
PAP (mm Hg)	19 ± 1	-	-	20 ± 2	21 ± 2#	21 ± 1#	20 ± 3
CVP (mm Hg)	7 ± 0	-	-	7 ± 0	7 ± 0	7 ± 0	7 ± 0
PCWP (mm Hg)	12 ± 1	-	-	11 ± 0	10 ± 0	11 ± 0	11 ± 0
SVRI (dyn·s·m·cm ⁻²)	3949 ± 267	-	-	3189 ± 312#	3739 ± 429	4321 ± 486	4730 ± 688
PVRI (dyn·s·m·cm ⁻²)	258 ± 23	-	-	409 ± 83	414 ± 91	479 ± 83#	404 ± 153

Table 1 depicts data of 6 controls and 6 TBC-1269-treated dogs. CO = cardiac output; CPB = cardiopulmonary bypass; CVP = central venous pressure; MAP = mean arterial blood pressure; PAP = mean pulmonary artery pressure; PCWP = precapillary wedge pressure; PVRI = pulmonary vascular resistance index; Reperfusion = empty beating state on CPB; SVRI = systemic vascular resistance index.

Values are mean ± SEM, # p < 0.05 vs. baseline.

CPB Techniques

After instrumentation, heparin (250 IU·kg⁻¹) was given intravenously for systemic anticoagulation followed by additional doses of 100 IU·kg⁻¹ given every 60 minutes throughout the experiment. Extracorporeal circulation was performed using a 14F arterial perfusion cannula placed into the right femoral artery and a two-stage venous cannula (36F and 46F) in the right atrium/inferior vena cava. The LV was vented with a 12F catheter inserted via the left atrium. The extracorporeal circuit (Model 7000, Sarns Inc., Ann Arbor, MI) and the membrane oxygenator (HVRF 3700, Cobe Cardiovascular Inc., Arvada, CO) were primed with 800 mL of Ringer's solution and 1000 IU of heparin. Body temperature was maintained at 37°C using a heat exchanger. CPB blood flow was maintained between 70 and 90 mL·kg⁻¹·min⁻¹ to maintain a mean systemic perfusion pressure of 40 to 70 mm Hg.

Experimental Protocol

After at least 30 minutes for stabilization following the completion of instrumentation, pre-ischemic hemodynamic values and LV pressure-volume loops were recorded. Myocardial samples for MWC determination were collected as described above. Arterial and coronary sinus blood samples were frozen at -20°C for later lactate determination (Sigma Diagnostics, St. Louis, MO). Experimental animals were subjected to CPB after intravenous administration of the selectin

analogue TBC-1269 (Texas Biotechnology Corp., Houston, TX), with a loading dose of 25 mg/kg body weight, followed by continuous infusion at 5 mg/kg/h throughout CPB duration. This dosing regimen was chosen on the basis of previous allometric pharmacokinetic modeling. Control animals received equal volumes of normal saline solution. We cross-clamped the aorta and initiated cardiac arrest by perfusing the coronary arteries with 15 mL/kg iced (4°C) HTK solution (Custodiol®, Köhler Chemie, Germany) into the aortic root at 100 cm H₂O (74 mmHg) for approximately 8-10 minutes. External myocardial cooling was accomplished by iced saline instillation into the pericardium. After 60 minutes the aortic cross-clamp was removed and the heart reperfused on normothermic CPB for 30 minutes. Thereafter, we weaned the dogs from CPB, removed all cannulas and discontinued the drug's infusion. Coronary sinus and arterial blood samples were collected simultaneously at 1 and 15 minutes during reperfusion (1'REP and 15'REP, respectively). Additionally, MWC was determined at 15 minutes of reperfusion. We repeated all measurements at 10, 30, 60, and 120 minutes after separation from CPB (10'pCPB, 30'pCPB, 60'pCPB, and 120'pCPB, respectively).

Statistical Analysis

All data presented are mean ± standard error of the mean. Data analysis was carried out using Statistica software (StatSoft

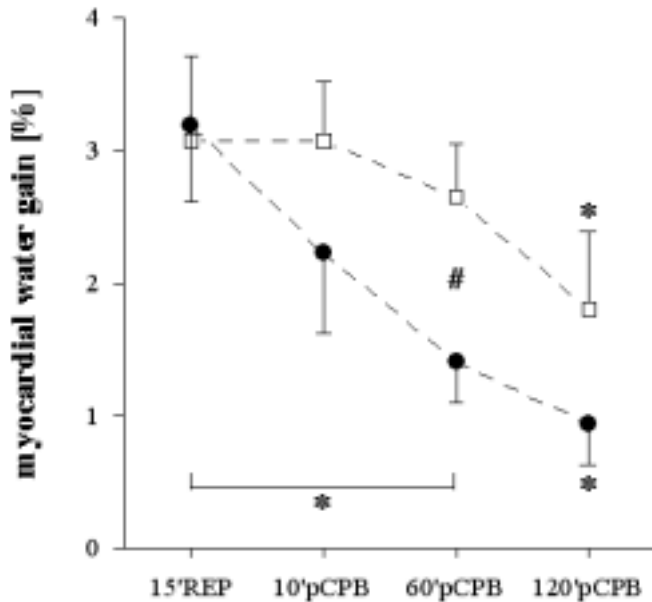


Figure 1. Myocardial water gain [%]. Both groups gained identical amounts of myocardial water following 60 minutes of cardioplegia. However, edema resolution in reperfused post-ischemic myocardium was significantly faster with the selectin analogue (I, $n = 6$) than with controls (o, $n = 6$). One hour after weaning from CPB myocardial water content was significantly reduced in the selectin analogue group, whereas controls still showed substantial edema. 15'REP, 15 minutes of "on-bypass reperfusion"; 'pCPB, minutes post-CPB ("off-bypass reperfusion"); * $p < 0.05$ vs. 15'REP; # $p < 0.05$ selectin analogue vs. controls.

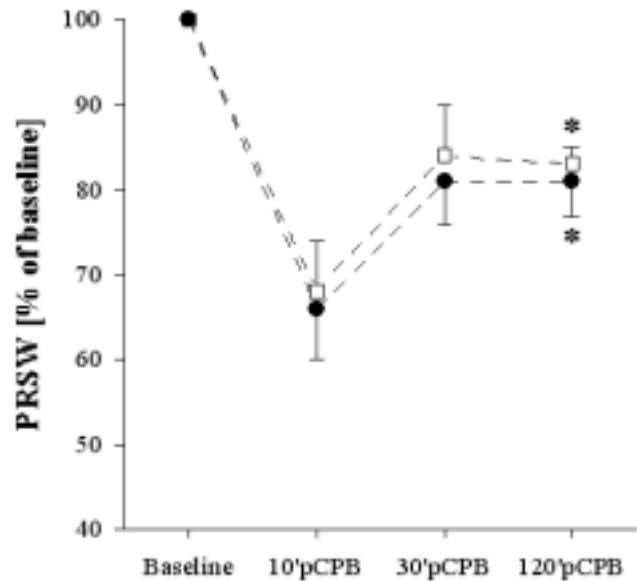


Figure 2. Global LV function. Preload recruitable stroke work (PRSW) after cardiopulmonary bypass. I, selectin analogue group ($n = 6$); o, controls ($n = 6$); 'pCPB, minutes post-CPB; * $p < 0.05$ vs. baseline.

Inc.). We examined the time courses of each measured parameter using analysis of variance (ANOVA) for repeated measures, and Turkey's was used for post hoc analysis of PRSW data between groups. Time point comparisons were made using the unpaired Student's *t* test. The level of significance was $p < 0.05$.

RESULTS

Data of twelve dogs were included for analysis: six in the control group and six in the selectin analogue group. Drug monitoring following bolus application showed plasma concentrations greater than 100 $\mu\text{g/mL}$, which had been demonstrated to effectively attenuate PMN sequestration in previous models of regional ischemia. Ventricular fibrillation requiring direct-current countershock developed in 85% of all dogs after cross-clamp removal and is expected with HTK cardioplegia. Therefore, defibrillation was not a criterion for exclusion for subsequent analysis. Hemodynamics are shown in Table 1 (●). We found no difference in hemodynamic data between TBC-1269 or control dogs. Additionally, there was no difference in net lactate production between groups throughout the experiment.

Myocardial Edema Formation

Both groups developed significant and similar amounts of myocardial edema following cardioplegia. At 15 minutes of reperfusion, there was no difference between the two groups in myocardial water gain (Figure 1, ●). TBC-1269 did not affect myocardial water gain during ischemia and in early

reperfusion. Thereafter, myocardial edema decreased in both groups, yet with better edema resolution ($p < 0.05$) and lower myocardial water content in TBC-1269 dogs ($p < 0.05$) at 60 minutes post-CPB. Compared to 15'REP, we found substantially less edema 120 minutes post-CPB for both groups ($p < 0.05$), but edema formation between groups did not attain statistical significance.

Myocardial Contractility

There were no differences between the control and treated groups in global LV function and contractile recovery, as measured by preload recruitable stroke work (PRSW) (Figure 2, ●). Although there was a trend toward better contractile function in the control group at 120 minutes post-CPB, mean values were not statistically different. For both groups, 120 minutes post-CPB values for PRSW were still lower compared to baseline ($p < 0.05$), yet we did not observe differences in contractile recovery between groups.

Myocardial PMN Infiltration

Comparisons of myeloperoxidase tissue activity in post-ischemic and reperfused myocardium revealed no significant reduction in PMN infiltration after two hours of reperfusion in treated dogs (Figure 3, ●). Despite a trend favoring less myeloperoxidase activity in the TBC-1269-treated dogs (0.31 ± 0.06 U/100mg tissue), myeloperoxidase activity was not different in controls (0.52 ± 0.14 U/100mg tissue). Therefore, administration of sLe^x analogue did not reduce PMN accumulation in the myocardium during ischemia and reperfusion.

DISCUSSION

The present study was undertaken to examine the effects of a selectin analogue on myocardial protection during CPB and

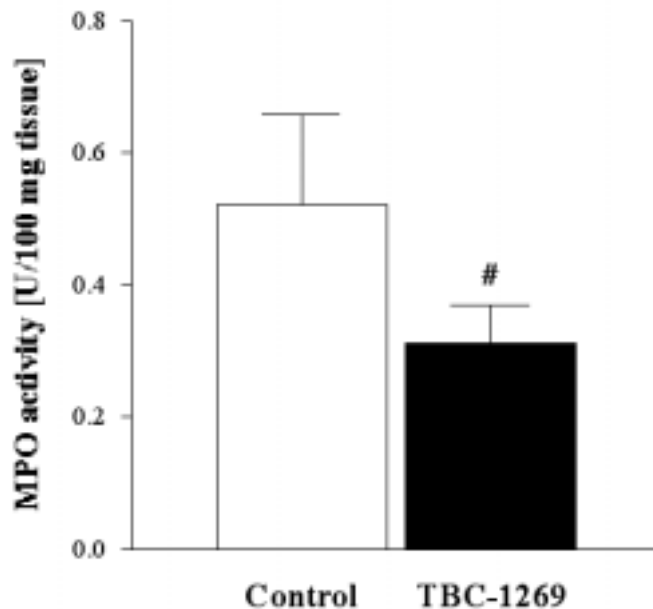


Figure 3. Myeloperoxidase activity. Left ventricular myeloperoxidase (MPO) tissue activity as marker for polymorphonuclear neutrophils (PMN) infiltration after CPB and cardioplegia followed by reperfusion in the presence (n, n = 6) or in the absence (o, n = 6) of the selectin analogue. # p = 0.24 TBC-1269 vs. controls.

crystalloid cardioplegia. Our findings show that administration of the selectin analogue TBC-1269 does not reduce PMN accumulation nor improve post-ischemic contractile recovery. Because we found no differences in myocardial water content between selectin inhibitor-treated animals and controls immediately off bypass and after 150 minutes of reperfusion, we therefore believe that sLe^x selectin-antagonists exhibit no ability to reduce the increase in myocardial microvascular permeability associated with CPB. However, we found enhanced edema resolution following cardioplegic arrest in TBC-1269 treated animals, indicating that PMN recruitment and leukocyte plugging are likely to be modified by pharmacological approaches that target adhesion molecules.

While the restoration of blood flow to ischemic myocardium may limit tissue injury, experimental evidence suggests that reperfusion itself may induce additional myocardial damage [Braunwald 1985, Hearse 1992]. Although there may be continuing controversy, studies aimed at the removal or inhibition of PMNs have provided evidence that PMNs play a major role in myocardial ischemia [Flynn 1996, Yamada 1998] and have proven that it is an effective means of reducing myocardial injury. Reintroduction of neutrophils in post-ischemic myocardium is succeeded by their activation before firm adhesion and transendothelial migration occurs [Anderson 1991]. In this multistep adhesion cascade, the PMN-endothelial cell interactions are mediated by selectins, a family of three structurally related calcium-dependent cell adhesion molecules. Selectins are expressed on the surface of activated vascular endothelium, platelets, and leukocytes, with their principle carbohydrate ligand being sLe^x [Lefer 1997]. PMN adherence is primarily the consequence of endothelial ligand interactions with these surface adhesion molecules.

Soluble sLe^x ligand administration has been shown to reduce tissue damage and PMN infiltration following regional ischemia and reperfusion [Buerke 1994, Han 1995, Palma-Vargas 1997]. If PMNs contribute to the pathogenesis of myocardial injury following CPB and cardioplegia, then their effective inhibition should mitigate this phenomenon. However, we did not observe such an effect.

We only examined the period during and immediately after CPB, and there are some data to suggest that 6-24 hours post-CPB may be a period of much greater impaired permeability due to upregulation of the endothelial adhesion molecules resulting in microvascular barrier injury. It is unlikely that the failure of neutrophil inhibition to attenuate post-ischemic dysfunction was due to an unfavorable distribution of variables that may affect contractile recovery. Control and treated dogs were quite similar with respect to body temperature, arterial pH, PO₂, hematocrit, and plasma electrolyte concentrations, as well as supplemental doses of thiopental required to maintain anesthesia. No negative inotropic effects were produced by the sLe^x analogue administration. It might be argued that the drug's failure to enhance functional recovery was due to an insufficient degree of selectin inhibition. This appears unlikely and is not supported by experimental myeloperoxidase activity data.

Measurements of myeloperoxidase, as an index of neutrophil infiltration in tissue, revealed a trend toward reductions in PMN sequestration due to TBC-1269 treatment in the reperfused myocardium. However, even if a reduction in PMN accumulation had been found, sonomicrometric data does not indicate better contractility to any extent, limiting the practical usefulness of the treatment. This may be due to a difference between our clinically applicable model of cardioplegia and other models of regional myocardial ischemia that did not include CPB and cardioplegia but where selectin analogues did attenuate infarct size. On the other hand, these findings may suggest that the endothelium of the coronary microvasculature is more sensitive to the effects of selectins and their antagonists than is myocardial contractility. However, numerous cellular adhesion molecules, including the integrins and the selectins, may be involved in PMN adhesion or transmigration during myocardial reperfusion [Verrier 1996]. It is quite conceivable that some of these mechanisms are redundant. If so, blockade or inhibition of one adhesion molecule could be offset by increased activation of a complementary adhesion molecule. TBC-1269 may not have inhibited PMN rolling. Since less than 1% of the rolling neutrophils adhere to post-ischemic capillaries, complete inhibition of rolling may be needed to prevent immigration of neutrophils [Kubes 1995]. In addition, P-selectins have been shown to be absolutely critical in the immediate neutrophil infiltration [Kanwar 1998], and one may speculate that a small fraction of selectins has the ability to produce the same degree of myocardial dysfunction following cardioplegia as the entire population of the selectin pool.

Another possible explanation of our results may be related to the calcium dependency of the drug and its time of administration. Since cell adhesion is mediated through calcium-dependent interactions of the selectins' carbohydrate recognition domain with cell surface oligosaccharides, the low cal-

cium concentration of HTK cardioplegia may inactivate the selectin analogue in the coronary arteries. Alternatively, cardioplegia perfusion per se may either wash out or dilute the drug in the coronary microvasculature. However, doses used in our study equal those used in previous studies in which protection was achieved [Palma-Vargas 1997]. Alterations of the drug's effectiveness by the CPB oxygenator and tubing cannot be ruled out, as TBC-1269 dosage in our study was comparable to other studies that did attenuate myocardial injury by selectin-antagonists but without extracorporeal circulation [Palma-Vargas 1997]. Such an acute model of cardioplegic arrest results in only minor myocardial damage, and healthy animals are apparently able to compensate for such a minor insult. Therefore, conclusions about ventricular function are limited in our study, and some caution is required in extending these findings to other models of myocardial injury. Finally, we cannot exclude the possibility that sLe^x is not the sole ligand for endothelial selectins on neutrophils.

CONCLUSION

Selectin adhesion molecule blockade is not effective in preserving myocardial function during CPB and crystalloid cardioplegia. The most likely interpretation of our results is that selectins do not play a prominent role in the pathogenesis of myocardial dysfunction in our experimental preparation. This conclusion, however, does not preclude an important role for PMNs as mediators of the irreversible cell damage associated with myocardial infarction as in the phagocytosis of necrotic material during the reparative phase of the healing process. Whether the failure to modify myocardial function was due to inadequate alteration of PMNs or to the possibility that PMNs do not cause myocardial dysfunction will require additional investigations.

Acknowledgments

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REVIEW AND COMMENTARY

1. Editorial Board Member GX21 writes:

The authors have drawn conclusions of “no effect” from the results of their statistical analysis. With a total sample size of 12 (n=6 in each group), they have low statistical power, and only relatively large changes will be detected as statistically significant. With a larger sample size, the changes the authors have observed would become statistically significant.

In the light of this, it would be helpful if the authors could comment on their limitations on what difference they considered to be clinically important, and what power they had to detect this difference in their study.

Authors' Response by H. Henning Sauer, MD:

As mentioned in the discussion “measurements of myeloperoxidase ... revealed a trend toward reductions in PMN sequestration due to TBC-1269 treatment in the reperfused myocardium. However, even if a reduction in PMN accumulation had been found, sonomicrometric data does not indicate better contractility to any extent, limiting the practical usefulness of the treatment. This may be due to a difference between our clinically applicable model of cardioplegia and other models of regional myocardial ischemia that did not include CPB and cardioplegia but where selectin analogues did attenuate infarct size.” In this study, we used a clinically relevant model of myocardial ischemia and reperfusion following CPB and cardioplegia. Even if, with a larger sample size, changes in MPO activity may become statistically significant, the failure of the selectin antagonist to improve cardiac function is the most important finding for the clinician.