Effect of Sialyl Lewis\(\text{X}\) Oligosaccharide on Myocardial and Cerebral Injury in the Pig

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Background. Although administration of the sialyl Lewis\(\text{X}\) oligosaccharide may reduce myocardial injury after ischemia–reperfusion, its effect on coronary and cerebral microvascular regulation and its clinical application during cardiac operation have not been examined.

Methods. Pigs were placed on normothermic cardiopulmonary bypass after 30 minutes of left anterior descending coronary artery occlusion. The hearts were then arrested with cold high potassium cardioplegia. After 1 hour the cross-clamp was removed and the pigs were weaned from cardiopulmonary bypass and perfused for an additional 1 hour. CY-1503 (a sodium salt of the sialyl Lewis\(\text{X}\) oligosaccharide, \(n = 6\)) was administered before reperfusion. Six other pigs received saline vehicle. Endothelium-dependent relaxation of precontracted coronary arterioles during ischemia followed by cardioplegia and cardiopulmonary bypass were improved with CY-1503, but the altered pattern of organ perfusion was not improved. Myeloperoxidase activity was increased in the heart after ischemia–cardioplegia and in the brain after cardiopulmonary bypass. CY-1503 reduced myeloperoxidase activity in both the myocardium and in the brain. Expressions of myocardial inducible isoform or constitutively expressed nitric oxide synthase were not altered in the heart.

Conclusions. Although the sialyl Lewis\(\text{X}\) oligosaccharide does reduce neutrophil infiltration and endothelial injury in the coronary and cerebral microcirculation after cardiopulmonary bypass, it does not have significant beneficial acute effects on organ perfusion or function in the myocardium or brain.

(During cardiovascular operations, extracorporeal circulation and myocardial ischemia–reperfusion leads to polymorphonuclear leukocytes (PMNs) accumulating in the heart, brain, and other organs in response to chemical mediators including cytokines (interleukins 1 and 8 and tumor necrosis factor \(\alpha\)), growth factors, leukotrienes, thromboxane, oxygen-derived free radicals, and chemotactic complement fragments including C5a. Expression of the neutrophil adhesion molecule P-selectin can be induced on the endothelial surface within 15 minutes after exposure of tissues to inflammatory mediators [1, 2]. This may initiate binding to sialyl Lewis\(\text{X}\) (SLe\(\text{X}\)) receptors on PMNs and enable PMNs to roll along the endothelial cells in postcapillary venules and other regions of the microcirculation. This initiates the adherence and transmigration of PMNs into the myocardium and other tissues, mediated primarily by integrins. The PMNs have been implicated in causing much of the inflammation and tissue injury occurring as a consequence of cardiopulmonary bypass (CPB) and acute ischemia. Recently, interest has increased in reducing myocardial ischemic injury and the inflammatory response to CPB with inhibitors of neutrophil adhesion and activation. The PMNs may produce leukotrienes, elastase, platelet-activating factor, and oxygen derived-free radicals that can seriously damage both the endothelium and adjacent tissues and increase permeability during migration of neutrophils. In addition, C5a activates PMNs during the migration process, leading to migration...
of PMNs and release of oxygen derived-free radicals and proteases and inflammatory mediators such as thromboxane [1–3]. These mediators may lead to further endothelial dysfunction and myocardial necrosis hours and perhaps days later.

In theory, inhibition or blockade of P- and L-selectins could prevent the initial adherence and rolling of PMN and preserve endothelial integrity and vascular and myocardial function after ischemia–reperfusion [4–6]. Analogs of the carbohydrate structure SLe\(^x\), which is expressed on the surface of PMN and binds to P- and E-selectins, show promise in inhibiting PMN adhesion. In vitro [7] and in vivo [8] studies have demonstrated that SLe\(^x\)-containing carbohydrates inhibit selectin-mediated PMN adherence to endothelium, attenuate myocardial necrosis, preserve endothelial function (of coronary arterial rings), and improved cardiac function after reperfusion. One of the analogs CY-1503 (a sodium carbohydrate salt SLe\(^x\), Cytel Corp, San Diego, CA), also could limit PMN tethering, adherence, and migration by blocking PMN interaction with these selectins.

The administration of SLe\(^x\)-containing carbohydrates during cardiac operation in which CPB is used has not been fully tested. The purpose of this study is to assess the effect of the SLe\(^x\) oligosaccharide CY-1503 on the heart and brain with particular emphasis on the microcirculation after acute myocardial ischemia, followed by cardioplegia and reperfusion under conditions of CPB.

This scenario may be encountered during emergency revascularization after acute coronary occlusion. If there is a beneficial role of CY-1503 during emergency cardiac procedures, it would be most important early after revascularization. Because low cardiac output and neurologic complications are leading causes of morbidity and mortality after cardiac operation, the effects of the SLe\(^x\) oligosaccharide on injury in the heart and brain were examined.

Material and Methods

Animal Preparation

Yorkshire pigs (20 to 25 kg) of either sex were premedicated with intramuscular ketamine (10 mg/kg) and anesthetized with \(\alpha\)-chloralose and urethane (60 mg/kg and 300 mg/kg intravenously initially, and 15 mg/kg and 60 mg/kg every 60 minutes as needed, respectively). Pigs were tracheally intubated and mechanically ventilated (Harvard Apparatus, Holliston, MA). In the control group (n = 6), a sternotomy was performed and the pig was heparinized (500 U/kg). The heart and brain were rapidly excised and immediately placed in a cold (0 to 4°C) Krebs’ buffer solution of the following composition (in mmol/L): 118.3 NaCl, 4.7 KCl, 2.5 CaCl\(_2\), 1.2 MgSO\(_4\), 1.2 NaH\(_2\)PO\(_4\), 25.0 NaHCO\(_3\), and 11.1 glucose.

In 12 pigs, after induction of anesthesia and tracheal intubation, fluid-filled catheters were introduced into the femoral artery, right internal jugular vein, and internal carotid artery. After a sternotomy was performed, purse-string sutures were placed in the distal ascending aorta and the right atrium. Internal carotid artery blood flow was measured with an ultrasonic transit-time flow probe (model 3RB; Transonic System Inc), that was placed proximal to a small catheter in the internal carotid artery. Instantaneous left anterior descending coronary artery (LAD) blood flow was measured with a 2.5-mm ultrasonic transit-time flow probe (model 2.5S; Transonic System Inc), that was placed around the mid-LAD. Probes were connected to flow monitors (model T206; Transonsics System Inc). A pair of ultrasonic crystals was implanted 10 to 15 mm apart and about 3 mm beneath the surface in the left ventricular subepicardium. The crystals were placed parallel to the long axis of the left ventricle. An 8F micromanometer-tipped catheter (model SPC-370; Millar Instruments, Houston, TX) was placed in the left ventricle through the apex for the measurement of left ventricular systolic pressure and rate of change of left ventricular (LV) pressure (LV dP/dt). LV dP/dt, the first derivative of LV pressure, was determined by differentiating the LV pressure signal over time. Pigs were heparinized (500 U/kg) and cannulated through the distal ascending aorta and the right atrium. The LAD just proximal to flow probe was then occluded for 30 minutes and then released before CPB. After stabilization of CPB, a LV vent was placed through the ventricular apex for decompression. A small cannula was inserted into the ascending aorta through a pursestring suture for the infusion of cardioplegic solution. An aortic cross-clamp was then placed and 300 mL of a cold (0 to 4°C) hyperkalemic crystalloid cardioplegic solution were infused into the aortic root at a pressure of 60 mm Hg. The heart was arrested for 60 minutes in total. The composition of hyperkalemic crystalloid cardioplegic solution was (in mmol/L) 121.0 NaCl, 25.0 KCl, 12.0 NaHCO\(_3\), and 11.1 glucose, in purified water. Saline slush was placed on the surface of the heart to provide topical hypothermia during the cross-clamp period. Myocardial temperature in the distribution of the LAD was measured with a probe and ranged from 4° to 10°C during the ischemic period. Infusion of the cardioplegic solution (150 mL) was repeated at 20-minute intervals for 60 minutes (two additional doses). The CPB was instituted using a bubble oxygenator (Bentley Bio-2, Baxter Healthcare Corp, Irvine, CA) and a roller pump. An arterial filter (Bentley Bio-1025, Baxter Healthcare Corp) was inserted into the circuit distal to the roller pump. Blood flow was maintained from 2.0 to 3.0 L/min (2.6 to 4.2 L · min\(^{-1} · m\(^{-2}\)) to maintain a mean perfusion pressure of 50 to 70 mm Hg. Systemic blood temperature was maintained at 37°C during the study. Arterial blood gases were obtained (model 1306; pH/Blood Gas Analyzer, Instrumentation Laboratory, Lexington, MA) before starting CPB and at approximately 20-minute intervals thereafter. Arterial blood gases were adjusted by ventilatory rate and tidal volume to maintain an arterial oxygen tension of more than 50 mm Hg, an arterial carbon dioxide tension between 30 and 45 mm Hg, and a pH between 7.35 and 7.45. For pH adjustment, alpha-stat pH management was used throughout the study. In 6 pigs, CY-1503 (40 mg/kg) was administered intravenously immediately before re-
moval of the aortic cross clamp. Six pigs underwent an identical procedure but received saline vehicle instead of saline containing the SLe\(^\circ\) oligosaccharide. The heart was then reperfused with normothermic blood from the bypass circuit and was kept decompressed with a left ventricular vent until a stable rhythm was obtained. In the event of ventricular fibrillation (all pigs), lidocaine (10 mg) was infused intravenously and the heart was defibrillated with 10 J after the myocardial temperature increased to more than 30°C. Pigs were weaned off from CPB by increasing left atrial pressure, and were then decannulated. After 60 minutes of reperfusion, the LAD-dependent subepicardial ischemic region in the left ventricle and a portion of the parietal lobe of the brain were excised rapidly and immediately placed in a cold Krebs’ buffer solution for the microvessel studies. Separate specimens were placed in liquid nitrogen in preparation for molecular studies.

All animals received humane care in compliance with the Beth Israel Deaconess Animal Care and Use Committee and the National Research Council’s Guide for the Care and Use of Laboratory Animals.

In vitro Microvessel Studies
Arterioles (70 to 177 μm in internal diameter) were dissected from the left ventricle myocardium and brain using a ×10–60 dissecting microscope (Olympus Optical, Tokyo, Japan). They were placed in a Plexiglass microvessel chamber, cannulated with dual glass micropipettes measuring 40 to 60 μm in diameter, and secured with 10-0 nylon monofilament sutures (Ethicon, Somerville, NJ). Oxygenated (95% O\(_2\)/5% CO\(_2\)) Krebs’ buffer solution warmed to 37°C was continuously circulated through the vessel chamber. The vessels were pressurized to 40 mm Hg in a no-flow state. With an inverted microscope (×40–200, Olympus CK2, Olympus Optical) connected to a video camera, the vessel image was projected onto a black and white television monitor. An electronic dimension analyzer (Living System Instrumentation, Burlington, VT) was used to measure internal lumen diameter.

Hemodynamic Measurements
The following parameters were measured every 30 minutes: LAD flow and pump flow (Q\(_{\text{CPB}}\) while on CPB), Mean arterial pressure, left ventricular systolic pressure, LV max dP/dt, internal juglar venous pressure (IJVP), and end-systolic segment length (EDSL) was measured at the onset of LV dP/dt and end-systolic segment length (ESSL) was measured at peak negative LV dP/dt. Cerebral vascular resistance (CVR) was calculated as follows: 80 × (ICAP – IJVP)/ICCA flow (dynes · sec · cm\(^{-5}\)). Measurements were obtained serially throughout the study.

Myocardial and Brain Myeloperoxidase Activity
Myocardial and brain samples were frozen in liquid nitrogen, and stored at −80°C until assayed. The myeloperoxidase (MPO) activity was measured as reported by Amsterdam and colleagues [9]. Ice cold, minced sections of the tissues were homogenized (10% wt/vol) with a Polytron homogenizer (Brinkman Instruments, Westbury, NY) in 50 mmol/L phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl-ammonium bromide (HTAB) for 15 seconds (×2). The homogenates were then sonicated for 15 seconds, and the supernatant containing the MPO enzyme was separated from the cellular debris by centrifugation at 20,000 g for 15 minutes (2°C). The appearance of a colored product from the MPO-dependent reaction of o-dianisidine and hydrogen peroxide (0.0005%) was spectrophotometrically detected using 100 μL of supernatant and 1.9 mL of the hydrogen peroxide and o-dianisidine in 50 mmol/L PBS (pH 6.0). Supernatant MPO activity was kinetically quantified on a temperature-controlled (25°C) spectrophotometer. The absorbance at 460 nm was recorded for several minutes, and the change in absorbance over 1 minute was measured. One unit of MPO activity was defined as that which degraded 1 mol hydrogen peroxide/min at 25°C. Assessment of this assay with isolated porcine neutrophils demonstrated a linear relationship (r = 0.92) between MPO and the number of cells such that 1 U of MPO activity correlated with 2.9 × 10\(^{6}\) neutrophils.

Microvessel Study Protocols
Relaxation responses of microvessels were examined after precontraction of coronary and brain microvessels with acetylcholine chloride and U46619, respectively, by 20% to 70% of the baseline diameter. Once the steady-state tone was reached, the dose responses to adenosine 5'-diphosphate (endothelium-dependent vasodilator, ADP) (10\(^{-9}\) to 10\(^{-4}\) mol/L) and sodium nitroprusside (endothelium-independent vasodilator, SNP) (10\(^{-6}\) to 10\(^{-4}\) mol/L) were examined in coronary and cerebral microvessels. All drugs were applied extraluminally. Measurements were made and recorded 2 to 3 minutes after the drug administration, when the response had stabilized. In noncontracted microvessels, contraction responses to phenylephrine (α\(_1\)-adrenoceptor agonist) (10\(^{-8}\) to 10\(^{-4}\) mol/L) were recorded 2 to 3 minutes after the drug administration. Responses to the protein kinase C activator phorbol 12-myristate 13-acetate (phorbol ester) (10\(^{-6}\) mol/L) were recorded every 10 minutes for 30 minutes after exposure. One to four interventions were performed on each vessel. The order of drug administration was random. The vessels were washed with Krebs’ buffer and allowed to equilibrate in a drug-free buffer for 15 to 30 minutes between interventions.

Reverse Transcriptase Polymerase Chain Reaction Analysis of cNOS and iNOS Expression
At the end of the study the tissue samples from the left ventricle were obtained, immediately frozen in a liquid nitrogen, and stored at −80°C. To examine myocardial...
mRNA of the constitutively expressed (cNOS) and the inducible isofrom of nitric oxide synthase (iNOS), reverse transcriptase polymerase chain reaction was performed. The cNOS and iNOS fragments were amplified by reverse transcriptase polymerase chain reaction from myocardium. Primers were designed based on the published cNOS [10] and iNOS sequence [11]. The primers of the sense 5'-AGACCCCTGGAAAGGAG-3' corresponding to bases 1,443 to 1,460, and the antisense 5'-TGTGTATCTGATTCCTCC-3' corresponding to bases 1,901 to 1,920 were used to amplify a 486-bp fragment of cNOS [10]. For iNOS, the primer of sense 5'-GCCTCGCTCTGGAAAGA-3' corresponding to bases 1,425 to 1,441, and the antisense 5'-TCCATGCAGACACCTT-3' corresponding to bases 1,908 to 1,924 were used to amplify a 500-bp fragment of cNOS [11]. An equal amount of total RNA was used for reverse transcriptase polymerase chain reaction from control and experimental groups. For quantification, glyceraldehyde-3-phosphate dehydrogenase was amplified from the same amount of RNA to correct for variation of different samples. The reverse transcriptase polymerase chain reaction products were loaded in 1.5% agarose gel, then scanned and measured.

Percent Tissue Water
The tissues from the left ventricle and parietal lobe of the brain were weighed, dried at 110°C for 8 hours, and then weighed again. Percent tissue water was calculated as (wet weight − dry weight)/wet weight × 100.

Polymorphonuclear Leukocytes and Platelet Induced Thromboxane Production
Porcine PMN and platelets were isolated as previously described [12]. Production of thromboxane from PMN and platelet cocultures were performed as previously described [12, 13]. Briefly, isolated porcine PMN (10^7/mL) and platelets (3 × 10^8/mL) were stimulated with porcine C5a (100 ng/mL) in a platelet aggregometer at 37°C for 5 minutes. The supernatant was removed after centrifugation and assayed by ELISA for thromboxane B2, the concentration of which was measured at 530 nm. The PMN and platelet suspensions were pretreated (4 minutes) with various concentrations of CY-1503 (0.1 to 10 μg/mL) before administration of porcine C5a.

Drugs
CY-1503 was obtained from Cytel Corporation (San Diego, CA). U46619, acetylcholine chloride, adenosine 5'-diphosphate, and sodium nitroprusside were obtained from Sigma Chemical (St. Louis, MO). Phenylephrine, clonidine hydrochloride, and phorbol 12-myristate 13-acetate were obtained from RBI (Natick, MA). Phorbol 12-myristate 13-acetate was dissolved in dimethyl sulfoxide and stored at −20°C. Other drugs used to examine vascular responses were dissolved in ultrapure distilled water. All solutions were prepared on the day of the study. Other drugs used in biochemical and molecular procedures are listed in the text.

Data Analysis
Microvessel relaxation responses to each agent were examined only once in each animal. Therefore, each animal served as one sample. The data were pooled from each dose response in each experimental group and an average was calculated. The relaxation responses were expressed as the percent relaxation of the acetylcholine or U46619-precontracted diameter (mean ± standard error of mean). The contraction responses were expressed as the percentage contraction of the baseline diameter. Comparisons of dose–response curves of experimental groups were performed by two-way analysis of variance with repeated measure design or factorial design, followed by Scheffe’s multiple comparison test post hoc. Student’s t test was used to compare changes in hemodynamic variables and gene expressions. Myocardial MPO data was compared by Kruskal-Wallis analysis of variance (ANOVA) followed by Neuman-Keuls test post hoc. Dunn’s nonparametric test post hoc was used for brain MPO data because data was not normally distributed. Statistical significance was taken at p values less than 0.05.

Results
Hemodynamic Parameters of the Heart and the Brain
Mean arterial pressure, LVSP, LV max dP/dt, and LAD blood flow did not show a statistical difference between groups (Table 1). Percent segmental shortening decreased after LAD occlusion (p < 0.05 versus baseline) and further deteriorated after reperfusion in both saline and CY-1503 groups. Mean arterial pressure and ICAP decreased after initiation of reperfusion. This was associated with a decrease in ICA blood flow and CVR (p < 0.05 versus baseline) after reperfusion. Again, there was no substantial difference between group (Table 1).

Myeloperoxidase Concentrations
Myeloperoxidase was measured to estimate neutrophil infiltration in the myocardium and in the brain. The MPO was increased in the myocardium in the saline group (0.47 ± 0.08 MPO U/g of tissue) compared to baseline levels before CPB of 0.03 ± 0.02 (p < 0.05 versus saline). In the CY-1503-treated pigs, the MPO concentration was significantly reduced to 0.17 ± 0.05 (p < 0.05 versus saline). Similarly, the MPO contentration in the brain was also increased in the brain after CPB (0.081 ± 0.060 MPO U/g of tissue) compared to baseline before CPB (0.002 ± 0.002, p < 0.05 versus saline). The MPO concentration was reduced in the CY-1503 group (0.005 ± 0.002, p < 0.05 versus saline) compared to baseline.

Coronary Microvessel Studies—Vessel Characteristics
Coronary microvessels ranged 70 to 177 μm in internal diameter, averaging 123 ± 8 in the control group, and 122 ± 10 and 106 ± 12 μm in the saline and CY-1503
groups, respectively. Percent precontraction after application of acetylcholine was 58% ± 5% in the control group, and 57% ± 7% and 53% ± 5% in the saline and CY-1503 groups, respectively. Mean concentrations of acetylcholine required to obtain these percent contractions were 0.7 × 10⁻⁷, 2.4 × 10⁻⁷, 1.2 × 10⁻⁷ mol/L in the control, saline, and CY-1503 groups, respectively.

Coronary Microvascular Endothelium-Dependent Relaxation

The relaxation response to the receptor-mediated endothelium-dependent vasodilator ADP were reduced (p < 0.01 versus control, two-way ANOVA) in saline group after 1 hour of reperfusion. The response to ADP was improved in the CY-1503 group, but only at the highest concentration examined (one-way ANOVA) (Fig 1). Relaxation responses to sodium nitroprusside were similar in all groups, indicating no alteration in the ability of the vascular smooth muscle to relax through the cyclic GMP pathway (Fig 2).

Contractile Responses In Vitro to Phenylephrine and Phorbol Ester

Phenylephrine caused only minimal change in dose responses in the control (−0.4 ± 0.4%, 10⁻⁵ mol/L), saline (0.4% ± 0.7%, 10⁻⁵ mol/L), and CY-1503 (0 ± 0%, 10⁻⁵ mol/L) groups. There was no significant difference in the responses between the groups.

As with phenylephrine, phorbol ester caused only minimal contraction of coronary arterioles in the control (−0.8 ± 0%, 30 minutes), saline (0.7% ± 0.4%, 30 minutes), and CY-1503 (0 ± 0%, 30 minutes) groups after 30

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### Table 1. Hemodynamics in Pig Heart and Brain

<table>
<thead>
<tr>
<th>Variables</th>
<th>Saline</th>
<th>CY-1503</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>Before CPB</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>82 ± 3</td>
<td>66 ± 4</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>97 ± 3</td>
<td>85 ± 3</td>
</tr>
<tr>
<td>dP/dt (mm Hg/sec)</td>
<td>1,210 ± 60, 1,100 ± 80</td>
<td>1,330 ± 180</td>
</tr>
<tr>
<td>%SS</td>
<td>100.0 ± 0</td>
<td>13.9 ± 20.2a</td>
</tr>
<tr>
<td>LAD flow (mL/min)</td>
<td>12.7 ± 2.0</td>
<td>....</td>
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<tr>
<td>ICA flow</td>
<td>81 ± 3</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>CVR</td>
<td>100.0 ± 0</td>
<td>90.9 ± 11.3</td>
</tr>
</tbody>
</table>

Data are mean ± standard error of the mean.

BL = baseline; before CPB = after 30 minutes of LAD occlusion and just before the commencement of CPB; 30 min REP = 30 minutes after reperfusion; 60 min REP = 60 minutes after reperfusion; MAP = mean peripheral artery pressure; LVSP = left ventricular systolic pressure; dP/dt = maximum rate of change of left ventricular pressure; %SS = percent of segmental shortening that is normalized to percent of a value obtained at baseline; LAD flow = left anterior descending coronary artery blood flow; ICA flow = internal carotid artery blood flow that is normalized to percent of a value obtained at baseline; CVR = cerebrovascular resistance that is normalized to percent of a value obtained at baseline.

*p < 0.05 versus baseline.

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**Fig 1. In vitro responses of precontracted porcine coronary microvessels to the endothelium-dependent vasodilator ADP from control hearts and hearts after ischemia–cardioplegia in the saline and CY-1503 groups. Responses are expressed as percent relaxation of acetylcholine-induced precontraction. (ADP-adenosine 5’-diphosphate.) 

**Fig 2. In vitro responses of precontracted porcine coronary microvessels to the endothelium-independent cyclic GMP-mediated vasodilator sodium nitroprusside (SNP) from control hearts and hearts after ischemia–cardioplegia in the saline and CY-1503 groups. Responses are expressed as percent relaxation of acetylcholine-induced precontraction.
minutes of exposure. In addition, there was no significant difference in the responses between the groups.

Cerebral Microvessel Studies—Vessel Characteristics
Brain microvessels ranged 95 to 171 μm in internal diameter, averaging 131 ± 6 in the control group, and 129 ± 9 and 140 ± 5 μm in the saline and CY-1503 groups, respectively. Percent precontraction after application of acetylcholine was 44% ± 3% in the control group, and 47% ± 3% and 45% ± 2% in the saline and CY-1503 groups, respectively. Mean concentrations of U46619 required to obtain these percent contractions were 0.1 × 10^{−7}, 0.4 × 10^{−7}, and 1.2 × 10^{−7} mol/L in the control, saline, and CY-1503 groups, respectively.

Cerebral Microvascular Endothelium-dependent Relaxation
Relaxation responses to ADP were slightly but significantly reduced (p < 0.05 versus control, two-way ANOVA) in the saline group after 1 hour of reperfusion, but only at the highest concentration examined (one-way ANOVA). The response to ADP in vessels from the CY-1503 group were similar to the control response (Fig 3). Relaxation responses to sodium nitroprusside were similar in all groups, indicating no alteration in the ability of the vascular smooth muscle to relax through the cyclic GMP pathway (Fig 4).

Cerebral Vascular Contraction to Phenylephrine and Phorbol Ester
Phenylephrine caused contraction responses in the control (−21.2% ± 1.2%, 10^{−5} mol/L), saline (−23.2% ± 2.1%, 10^{−5} mol/L), and CY-1503 (−21.9% ± 1.8%, 10^{−5} mol/L) groups. There was no significant difference in the responses between the groups.

Likewise, phorbol ester caused potent contraction responses in the control (−35.7% ± 2.7%), saline (−34.9% ± 2.3%), and CY-1503 (−28.0% ± 2.3%) groups after 30 minutes of exposure (p < 0.05 CY-1503 versus control and saline).

C5a-Stimulated Thromboxane Production
Cocultures of PMNs and platelets synthesized thromboxane in response to stimulation with C5a, as measured by...

iNOS and cNOS Expression
The expression of neither iNOS or cNOS was altered in the heart after ischemia–cardioplegia followed by reperfusion. In addition, CY-1503 had no effect on the expression of iNOS or cNOS (Fig 5).

Wet/Dry Ratios
Myocardial wet/dry ratios were 4.21 ± 0.07, 5.63 ± 0.60 (p < 0.05 versus control), and 5.63 ± 0.48 (p < 0.05 versus control) in the control, saline, and CY-1503 groups, respectively. There was no difference between the saline and CY-1503 groups. In brain tissue, wet/dry ratios were 4.56 ± 0.04, 4.55 ± 0.12, and 4.57 ± 0.07 in the control, saline, and CY-1503 groups, respectively. No increase in wet/dry weight ratio was observed, nor was a difference observed between groups.

Fig 3. In vitro responses of precontracted porcine brain microvessels to the endothelium-dependent vasodilator ADP from control hearts and hearts in the saline and CY-1503 groups after ischemia–cardioplegia. Responses are expressed as percent relaxation of U46619-induced precontraction. (ADP-adenosine 5’-diphosphate.) *p < 0.05 versus control.

Fig 4. In vitro responses of precontracted porcine brain microvessels to the endothelium-independent cyclic GMP-mediated vasodilator sodium nitroprusside (SNP) from control hearts and hearts in the saline and CY-1503 groups after ischemia–cardioplegia. Responses are expressed as percent relaxation of U46619-induced precontraction.

Fig 5. Representative reverse transcriptase polymerase chain reaction products of cNOS and iNOS in the porcine heart, shown in GAPDH-stained 1.5% agarose gel for amount of RNA control.
the stable metabolite of thromboxane A<sub>2</sub>, B<sub>2</sub>. CY-1503 did not inhibit the production of thromboxane (at CY-1503 [μg/mL] 0, 0.1, 1.0, and 10.0 thromboxane [ng/mL] was 6.2 ± 1.6, 9.8 ± 2.8, 6.7 ± 1.6, and 6.9 ± 1.5, respectively). Even concentrations of CY-1503 up to 500 μg/mL were not effective in inhibiting thromboxane production in the PMN–platelet cocultures in response to C5a.

Comment

In this study, myocardial ischemia followed by cardioplegia–reperfusion was associated with impaired endothelium-dependent relaxation and markedly reduced myocardial contractile function. Administration of the Sle<sup>+</sup> oligosaccharide reduced the reperfusion injury and neutrophil accumulation in the coronary microcirculation as may occur after acute coronary occlusion. However, the Sle<sup>+</sup> oligosaccharide did not affect other indices of cardioplegia or CPB-induced injury. In addition, CY-1503 did not affect the synthesis of the inflammatory mediator thromboxane in cocultures of PMNs and platelets in response to C5a. Thus, with the exception of improving endothelium-dependent relaxation in coronary artery rings and attenuated PMN adherence and migration into extravascular tissues [14, 15], this basal loss of nitric oxide accelerates PMN adherence and migration into extravascular tissues. However, neither cNOS nor iNOS expression was altered. This suggests that the altered endothelium-dependent relaxation of the microcirculation in the heart was not related to altered expression of the constitutive isoform of NOS. Rather, reduced activity of the enzyme or a defect in signal transduction between the receptor and the second messenger mechanism is more likely the cause of reduced endothelial function. Induction of iNOS has been implicated in causing much of the inflammation attributable to cytokines, oxygen-derived free radicals, and macrophages. However, CPB and ischemia–cardioplegia and reperfusion times were relatively short in this study and probably too brief to cause significant expression of iNOS. Thus, the inflammatory effects observed in this study are due to factors other than induction of NOS expression.

The reduced endothelium-dependent relaxation of coronary and brain microvessels to ADP during cardiac arrest and CPB were slightly improved with the administration of CY-1503. Several possibilities could account for the small beneficial effect of Sle<sup>+</sup> oligosaccharide on coronary and cerebral vascular reactivity and its failure to improve myocardial contractility in this study. First, the CPB circuit itself activates PMNs and may lead to endothelial dysfunction in the coronary [20, 21] and brain [22] microcirculation. It is also possible that the timing of CY-1503 administration is critical. Much of the injury may have been initiated before administration of the drug. We selected a time that would be similar to what would likely be performed clinically. Furthermore, Ito and colleagues [23] reported that resident cardiac mast cells, rather than just neutrophils, release cytotoxic products. Both of these products may mediate C5a-induced cardiac dysfunction. Furthermore, the C5b-9 terminal membrane attack complex may cause much of the cardioplegia- and CPB-induced endothelium dysfunction observed soon after initiation of reperfusion [24] or commencement of CPB [25]. Direct complement-mediated injury would not be affected by the administration of the Sle<sup>+</sup> oligosaccharide. Finally, adhesion molecules other than those inhibited by sialyl Lewis<sup>a</sup> oligosaccharide could account for most of the damage in the heart and brain of pigs.

Interestingly, the contraction responses of brain microvessels to both phenylephrine and the PKC activator phorbol ester were stronger than the respective responses observed in coronary microvessels. This suggests that porcine brain microvessels tend to be more contractile to vasoconstrictor agents that act through the α<sub>1</sub>-adrenoceptor–PKC pathway than are coronary microvessels. This may have implications regarding the differential changes in organ perfusion during administration of pressor agents such as phenylephrine. In both organs, minimal alterations to responses of agents that operate in the α<sub>1</sub>-adrenoceptor–PKC pathway were observed after ischemia–cardioplegia or CPB.
Limitations and Clinical Implications

There are several significant limitations of the present study. First, a porcine model of myocardial ischemia, cardioplegia, and CPB was used. It is possible that greater effects of the SLeα oligosaccharide would have been apparent using another animal model. Second, the length of reperfusion time in this study may be too brief to determine the effect of CY-1503. However, an early neutrophil accumulation in myocardium and expression of selectins has been reported within 1 hour of reperfusion. Therefore, we do not necessarily consider that 1 hour of reperfusion is a major limitation of the study. Because the concentration of CY-1503 inhibited neutrophil infiltration in the heart, the blood concentration is likely adequate to bind to P-selectin and other selectin adhesion molecules.

In conclusion, blocking P-selectin-mediated leukocyte-endothelium interaction with the SLeα oligosaccharide does prevent injury to the coronary and cerebral microcirculation by inhibiting PMN infiltration, but it does not have a striking impact as a potential strategy to prevent reperfusion injury to the heart and brain during urgent cardiac operations.

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References