Effects of Supplemental Sialyl Lewis\textsuperscript{x} Analogue During Warm Blood Cardioplegia

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Summary: Effects of supplemental Sialyl Lewis\textsuperscript{x} analogue, a major ligand for all three selectin family members, during warm blood cardioplegia were assessed in the blood perfused isolated rat heart. The isolated hearts were arrested for 60 min with warm blood cardioplegia given at 20-min intervals. This was followed by 60 min of reperfusion. The hearts were divided into the following two groups according to the supplemental drugs added to the cardioplegic solution. The control group (n=6) received standard warm blood cardioplegia. The Sialyl Lewis\textsuperscript{x} analogue group (n=6) received warm blood cardioplegia supplemented with Sialyl Lewis\textsuperscript{x} analogue (60 \(\mu\)g/ml). Cardiac function, endothelial function, myocardial metabolism and myocardial myeloperoxidase activity were assessed before and after cardioplegic arrest. Left ventricular developed pressure and dp/dt were significantly (p<0.05) greater and -dp/dt was significantly (p<0.05) lower in the Sialyl Lewis\textsuperscript{x} analogue group than the control group during reperfusion. Coronary flow at 15 min of reperfusion and NO production, when acetylcholine chloride was added were significantly (p<0.05) greater in the Sialyl Lewis\textsuperscript{x} analogue group than the control group. Myeloperoxidase activity was significantly (p<0.05) lower in the Sialyl Lewis\textsuperscript{x} analogue group than the control group. The results suggest that selectin-mediated endothelial-leukocyte interactions may play an important role in myocardial ischemia and reperfusion injury. Supplementation of Sialyl Lewis\textsuperscript{x} analogue during warm blood cardioplegia may provide superior myocardial protection by suppressing leukocyte-endothelial interaction during early reperfusion period.

Key words: Sialyl Lewis\textsuperscript{x}, cardioplegia, myocardial reperfusion injury, endothelial function, myocardial myeloperoxidase activity

INTRODUCTION

Aortic cross-clamping and cardioplegic arrest are essential techniques for cardiac operations, however, readmission of coronary flow by aortic declamping has been reported to induce a paradoxical extension of ischemic damage during cardioplegic arrest, the so-called ischemia-reperfusion injury [1]. Reversible and irreversible damage to the myocardium and endothelium induced by this phenomenon have been reported to be associated with a delayed recovery of myocardial metabolism and cardiac function [1,2]. Activated neutrophils have been implicated as causing tissue destruction during reperfusion [1-3]. Although the rate of neutrophil recruitment into the ischemic myocardium is greatest within the first hour of reperfusion, it is likely that the cumulative influx of neutrophils proceeds for several additional hours [4]. Adhesion of neutrophils to the endothelial cells is one of the important steps that leads to neutrophil infiltrate into the myocardium and subsequent reperfusion injury [5]. The adhesion of neutrophils consists of multiple steps facilitated by several species of adhesion molecules present both on neutrophils and endothelial cells [1,5]. In the inflammatory response, neutrophils initially interact with endothelial cells via selectins (E-, P-, and L-selectin), which are responsible for tethering neutrophils [5]. Of the three mem-
bers of the selectin family, P-selectin, which is normally present in the Weibel-Palade body of endothelial cells and the a-granule of platelets, is rapidly translocated to the cell membrane upon stimulation by thrombin, histamine or reactive oxygen species [6]. E-selectin is expressed on endothelial cells several hours after stimulation by interleukin-1, or tumor necrosis factor-a [7]. L-selectin is constitutively expressed on leukocytes [8]. After the tethering stage, neutrophils adhere firmly to endothelial cells with the interactions between members of the integrin (CD11a/CD18, CD11b/CD18) and immunoglobulin superfamilies (intercellular adhesion molecule-1, -2; ICAM-1, -2), causing neutrophils to migrate into inflammatory sites [5]. Adhered or migrated neutrophils undergo activation and release several toxic substances including reactive oxygen species and proteolytic enzymes that subsequently cause tissue injury [1-3,5]. The important ligands for selectins are considered to be the sialylated, fucosylated carbohydrates such as Sialyl Lewis [9]. Several investigators have demonstrated that a soluble form of Sialyl Lewis-containing oligosaccharide attenuated myocardial ischemia reperfusion injury by inhibiting selectin-ligand interactions [10,11]. With regard to the technique of the cardioplegia, warm (30-37 °C) blood cardioplegia has been suggested to better preserve myocardial metabolism and cardiac function than cold blood cardioplegia by preventing endothelial dysfunction [12,13]. Therefore, it is expected that supplementation of Sialyl Lewis-containing oligosaccharide to warm blood cardioplegia may provide superior myocardial protection by preventing the ischemia-reperfusion injury. In the present study, we used a blood-perfused, isolated rat heart preparation to investigate the influences of Sialyl Lewis-containing oligosaccharide supplementation during warm blood cardioplegia on cardiac function, myocardial metabolism, and leukocyte-endothelial cell interaction during reperfusion.

MATERIALS AND METHODS

Blood-perfused isolated rat heart preparation

All animals in this study received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health (NIH Publication No. 86-23, revised in 1985). The blood-perfused isolated rat heart preparation was a modification of the paracorporeal rat heart preparation used by Walters and colleagues [14]. A large SD rat (350-400 g) was anesthetized with sodium pentobarbital (50 mg, intramuscularly) and anticoagulated with heparin (1000 IU/kg, intravenously). The rat was placed supine on a heating plate (HP-4530; Iuchi, Osaka, Japan) which was maintained at 38 °C. Tracheotomy was then performed and the rat was ventilated and oxygenated with a ventilator (SAR-830/A; CWE Inc., Ardmore, PA, USA) in order to maintain arterial PO2 above 200 mmHg and PCO2 within 30-40 mmHg. Teflon catheters (Angiocath, 24-gauge, Becton Dickinson Vascular Access, Sandy, UT) were positioned in the left femoral vein (for the administration of fluids and the return of blood collected from the isolated perfused heart) and the left femoral artery (for supplying arterial blood to the isolated heart). The right femoral artery was also cannulated with the same catheter for monitoring systemic arterial pressure of the support rat. During the experiments the support rat’s mean arterial blood pressure was maintained between 60-70 mmHg. The heart donor rat was anesthetized with 30 mg of intramuscular sodium pentobarbital and was anticoagulated with heparin (1000 IU/kg, intravenously). The heart was excised rapidly and was perfused in the Langendorff mode with blood (36-37 °C) through a thermostatically controlled delivery line from the support rat by a peristaltic pump (NBM-1000, Mera, Tokyo, Japan). Perfusion was controlled with the aid of a pump speed driver that was designed to adjust the flow rate continuously to maintain a constant aortic pressure of 80 mmHg. The interval between isolation of the heart and initiation of coronary perfusion was less than 30 sec in all experiments. The coronary venous blood of the isolated heart was collected and returned to the support rat by means of the pump. The perfusion circuit was primed with 20 ml heparinized blood collected from another same strain rat and 20 ml lactated Ringer’s solution. The donor rat’s blood aspirated from the pericardium through a polypropylene filter by a syringe was also added to the perfusion circuit. Hematocrit of the perfusate was maintained between 25-30% and pH of the perfusate was maintained between 7.35 and 7.45 by administration of sodium bicarbonate. Surfaces of all glass components in contact with blood were siliconized to minimize the activation of platelets and leukocytes.
**Experimental protocol**

After a 20-minute equilibrium period, the isolated heart was arrested with warm blood cardioplegia (37 °C, 5 ml/min, 2 min). The cardioplegic solution was prepared by mixing four parts of an oxygenated blood to each part of crystalloid solution (Femes solution) by means of a syringe pump (CFV-3100, Nihon Kohden, Tokyo, Japan). The second and third dose (37 °C, 5 ml/min, 1 min) of cardioplegia were given at 20-minute intervals. The last dose was given immediately before reperfusion. After 60 min of cardioplegic arrest, reperfusion was begun with 37 °C oxygenated blood. After 60 min of reperfusion, acetylcholine chloride was added to the coronary perfusate so as to achieve a final concentration of $1 \times 10^{-8}$ mol/L. The isolated hearts were divided into the following two groups according to the type of cardioplegia received. In the control group (n=6), standard warm blood cardioplegia was given. In the Sialyl Lewis$^x$ analogue group (n=6), Sialyl Lewis$^x$ analogue (purchased from DEXTRA LABORATORIES, LTD, UK) was added to the cardioplegia so that the concentration of Sialyl Lewis$^x$ analogue was 60 μg/ml (Fig. 1).

**Measurements**

A bipolar pacing electrode was placed on the right ventricle and the heart was paced at 360 beats per minute. A latex balloon, inserted into the left ventricular cavity through an incision in the left atrium, was connected by a short polyvinyl tube to a pressure transducer (UK4006SB<TW>, Baxter, Tokyo, Japan); the signal was amplified, converted digitally and displayed on a computer screen using Mac LAB system (AD Instruments, NSW, Australia). The balloon was large enough that no measurable pressure was generated by the balloon itself over the range of left ventricular volumes used during the experiment. Left ventricular function was evaluated during isovolumic contraction by stepwise inflation of the intraventricular balloon (0.05 ml, 0.1 ml, 0.15 ml, 0.2 ml). Left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), positive maximum rate of left ventricular pressure rise (dp/dt, mmHg/s) and negative maximum dp/dt (-dp/dt, mmHg/s) were measured and recorded (Mac LAB system) after a 20-minute equilibrium period, at 15 min and 60 min of reperfusion. The coronary blood flow (C. flow) was measured by timed collection of the coronary sinus blood (Fig. 1).

Blood samples were obtained and assayed for the determination of MB isoenzyme of creatine kinase (CK-MB), thiobarbituric acid reactive substances (TBARS), neutrophilic elastase (ELAS), myocardial oxygen consumption (MVO$_2$), and nitric oxide production (NO prod.). Coronary sinus blood samples for the determination of CK-MB, TBARS, and ELAS levels were collected after a 20-minute equilibrium period, at 15 min and 60 min of reperfusion (Fig. 1).

Arterial and coronary sinus blood samples for the determination of MVO$_2$ and NO prod. were collected simultaneously after a 20-minute equilibrium period, and then at 3, 15 and 60 min of reperfusion. Additional blood samples were collected after the administration of acetylcholine chloride for the determination of NO prod. (Fig. 1).

CK-MB levels were measured with a chemiluminescent immunoassay (Type II Chemilumi-Analyzer; Ciba Corning, Medfield, MA), TBARS levels were measured with a thiobarbituric acid reaction (RF-5000; Shimadzu, Tokyo, Japan), and ELAS were measured with an enzyme immunoassay (Plateleader, multiscanbiclomatics, Labsystems, Finland). The samples were immediately centrifuged at 4 °C at 3000 rpm for 15 min and were stored at −80 °C until analysis.
Blood samples for the determination of NO concentration were centrifuged immediately at 3000 rpm for 5 min at 4 °C and the serum was immediately mixed with methanol and centrifuged at 15000 rpm for 60 min at 4 °C. The supernatant was measured by Griess reaction (ENO-20, Eicom Co., Ltd., Kyoto, Japan). NO concentration was determined by re-converting its oxidation end-products, nitrite (NO$_2^-$) and nitrate (NO$_3^-$). NO prod. and MVO$_2$ were calculated as C. flow multiplied by the difference between the coronary arterial and coronary venous content. Oxygen content was calculated by the following formula: Oxygen content (vol. %) = oxygen saturation (%) × Hb (g/dl) × 0.013.

The activity of myeloperoxidase (MPO), an enzyme virtually exclusive for neutrophils, was measured in myocardial tissue in accordance with the method described by Henson et al. [15] and Mullane et al. [16]. MPO activity was considered as an index of neutrophil accumulation in the heart. At the end of the reperfusion period, the heart was perfused with physiological saline until the heart was cleared of blood. Left ventricular myocardial samples for the measurement of MPO were excised and frozen in liquid nitrogen, and stored at −80 °C until assayed. Myeloperoxidase was extracted from homogenized tissue by suspending the tissue in 50 mM potassium phosphate buffer, pH 6.0 containing 0.5% hexadecyltrimethylammonium bromide (HTAB). The samples were then sonicated for 30 sec, five times, and freeze-thawed three times with dry ice-methanol. Suspensions were then centrifuged at 40,000 g for 15 min and the resulting supernatant or pellet were assayed. Myeloperoxidase activity was assessed by adding 40 μl supernatant to 200 μl buffered reagent (Hank’s BSA: 125 mM potassium phosphate buffer, pH 6.2=3:2) and 10 μl H$_2$O followed by 20 μl, 22.5 mM o-dianisidine and 20 μl, 15 mM H$_2$O$_2$, both in distilled water with terminal concentration of o-dianisidine to reach 1.5 mM, H$_2$O$_2$ to 1.0 mM. The change in absorbance at 460 nm was measured with COBAS FARA (Roche diagnostics, Switzerland). One unit of MPO activity was defined as that degrading one micromole of peroxide per minute at 30 °C. The MPO activity was normalized to the total protein concentration as determined by the method of Lowry et al. [17].

**Statistical analysis**

Statistical analysis was performed with Stat View J-5.0 software (SAS Institute, Cary, NC). All data are expressed as the mean ± the standard error of the mean. One-way or two-way repeated-measures analysis of variance was used to test the effect of cardioplegia group and time on cardiac function, CKMB, TBARS, ELAS, MVO$_2$, C. flow, NO prod, and MPO. When analysis of variance indicated a significant effect of cardioplegia group or time (p<0.05), the differences were specified with Scheffe's test for between-group comparison. Significance was assumed at a probability level of less than 0.05.

**RESULTS**

**Cardiac function**

There were no significant differences in the base line cardiac function between the groups (Figs 2-5). Marked decreases in LVDP and dp/dt were observed in the control group during reperfusion and the levels were significantly (p<0.05) lower than those in the Sialyl LewisX analogue group at 15 min and 60 min of reperfusion (Figs 2 and 4). The Sialyl LewisX analogue group resulted in significantly (p<0.05) less dp/dt than the control group at 30 min and 60 min of reperfusion (Fig. 5). No significant difference was found in LVEDP between the groups at any time (Fig. 3).

**Myocardial oxygen consumption (MVO$_2$)**

There was no significant difference in the base line MVO$_2$ between the groups (Fig. 6). The levels of...
EFFECTS OF SIALYL LEWISx DURING CARDIOPLEGIA

Fig. 3. LVEDP observed at the left ventricular balloon volume of 0.05 ml. No significant difference was found in LVEDP between the groups at any time. Control : control group; SLX : Sialyl Lewisx analogue group; LVEDP : left ventricular end-diastolic pressure; base : before ischemia; 15 m : 15 min of reperfusion; 60 m : 60 min of reperfusion Bar heights represent mean; error bars represent SE

Fig. 4. dp/dt observed at the left ventricular balloon volume of 0.05 ml. Marked decrease in dp/dt was observed in the control group during reperfusion and the levels were significantly (p<0.05) lower than those in the Sialyl Lewisx analogue group at 15 min and 60 min of reperfusion. Control : control group; SLX : Sialyl Lewisx analogue group; dp/dt : positive maximum rate of left ventricular pressure rise; base : before ischemia; 15 m : 15 min of reperfusion; 60 m : 60 min of reperfusion Bar heights represent mean; error bars represent SE

Fig. 5. -dp/dt observed at the left ventricular balloon volume of 0.05 ml. The Sialyl Lewisx analogue group resulted in significantly (p<0.05) less -dp/dt than the control group at 15 min and 60 min of reperfusion. Control : control group; SLX : Sialyl Lewisx analogue group; -dp/dt : negative maximum dp/dt; base : before ischemia; 15 m : 15 min of reperfusion; 60 m : 60 min of reperfusion Bar heights represent mean; error bars represent SE

MVO2 tended to be greater in Sialyl Lewisx analogue group than those in control group (9.3±1.2 ml/min vs. 5.8±1.1 ml/min; p=0.051) at 3 min of reperfusion (Fig. 6).

Endothelial function

There were no significant differences in the levels of C. flow and NO prod. between the groups during base line measurements (Figs 7 and 8). Marked decreases in C. flow were observed in the control group during reperfusion and the levels were significantly less than those in the Sialyl Lewisx analogue group (1.9±0.1 ml/min vs. 2.6±0.2 ml/min; p=0.002) at 15 min (Fig. 7). Although there were no significant differences in endothelial NO release between the groups at any time, the administration of acetylcholine chloride to the coronary perfusates at 60 min of reperfusion resulted in significantly greater NO prod. in the Sialyl Lewisx analogue group than in
Fig. 7. Marked decreases in C. flow were observed in the control group during reperfusion and the levels were significantly (p<0.05) less than those in the Sialyl LewisX analogue group at 15 min and there was a tendency to be less in the control group. Ach.: acetylcholine chloride was administered to the coronary perfusate so as to achieve a final concentration of $1 \times 10^{-8}$ mol/L.; Control: control group; SLX: Sialyl LewisX analogue group; C. flow: coronary blood flow; base: before ischemia; 3 m: 3 min of reperfusion; 15 m: 15 min of reperfusion; 60 m: 60 min of reperfusion. Bar heights represent mean; error bars represent SE.

Fig. 8. Although there were no significant differences in endothelial NO release between the groups at any time, the administration of acetylcholine chloride to the coronary perfusates at 60 min of reperfusion resulted in significantly greater NO prod. in the Sialyl LewisX analogue group than in the control group (p<0.05). Ach.: acetylcholine chloride was administered to the coronary perfusate so as to achieve a final concentration of $1 \times 10^{-8}$ mol/L.; Control: control group; SLX: Sialyl LewisX analogue group; NO prod.: nitric oxide production; base: before ischemia; 3 m: 3 min of reperfusion; 15 m: 15 min of reperfusion; 60 m: 60 min of reperfusion. Bar heights represent mean; error bars represent SE.

MB isoenzyme of creatine kinase (CKMB), and thiobarbituric acid reactive substances metabolism (TBARS), neutrophilic elastase (ELAS)

CKMB, TBARS, and ELAS levels in coronary sinus blood before cardiac arrest and during reperfusion are shown in Figs 9-11. There were no significant differences in CKMB, TBARS, and ELAS levels between the groups at any time (Figs 9-11).

Fig. 9. There were no significant differences in CK-MB levels between the groups at any time. Control: control group; SLX: Sialyl LewisX analogue group; CK-MB: MB isoenzyme of creatinine kinase; base: before ischemia; 15 m: 15 min of reperfusion; 60 m: 60 min of reperfusion. Bar heights represent mean; error bars represent SE.

Fig. 10. There were no significant differences in TBARS levels between the groups at any time. Control: control group; SLX: Sialyl LewisX analogue group; TBARS: thiobarbituric acid reactive substances; base: before ischemia; 15 m: 15 min of reperfusion; 60 m: 60 min of reperfusion. Bar heights represent mean; error bars represent SE.
EFFECTS OF SIALYL LEWISX DURING CARDIOPLEGIA

There were no significant differences in ELAS levels between the groups at any time. Control: control group; SLX: Sialyl LewisX analogue group; ELAS: neutrophil elastase; base: before ischemia; 15 m: 15 min of reperfusion; 60 m: 60 min of reperfusion
Bar heights represent mean; error bars represent SE

There was significantly less MPO activity in the Sialyl LewisX analogue group than in the control group (p<0.05). Control: control group; SLX: Sialyl LewisX analogue group; MPO: myocardial myeloperoxidase activity
Bar heights represent mean; error bars represent SE

**DISCUSSION**

Adhesion of neutrophils to the endothelial cells is one of the important steps that lead neutrophil to infiltrate into the myocardium and contribute to reperfusion injury [1,5]. The adhesion of neutrophils consists of multiple steps facilitated by several species of adhesion molecules present both on neutrophils and endothelial cells [1,5]. Studies using monoclonal antibodies against adhesion molecules, that are present on neutrophils and endothelial cells, demonstrated that the anti-adhesion therapy was beneficial at least at the experimental level [18-21]. In a feline model, anti-L-selectin monoclonal antibody and anti-P-selectin monoclonal antibody attenuated myocardial necrosis [18,19]. In a canine model, anti-CD11b/CD18 monoclonal antibody and anti-ICAM-1 monoclonal antibody also reduced infarct size [20,21]. The use of antibodies to block adhesion molecules in vivo, however, may be potentially limited by the deleterious effects of the antigen/antibody complexes which are formed at the endothelial cell surface, possibly through Fc receptor mechanisms. The phenomenon may result in adverse long-term outcomes similar to vascular rejection processes and accelerate atherosclerosis as seen in cardiac transplant recipients [22]. A different approach to prevent neutrophil adhesion is the development of soluble substances that saturate the adhesion molecules. Of these, Sialyl LewisX, a small sugar moiety, has low antigenicity and is a major ligand for all three selectin family members [9]. It has been proven that Sialyl LewisX was even more cardioprotective than individual monoclonal antibodies directed to a single selectin [10,11]. In isolated neonatal lamb hearts, Schermerhorn and colleagues have demonstrated that Sialyl LewisX analogue (40 mg/l) added to blood perfusate before ischemia reduced myocardial neutrophil accumulation and contractile dysfunction after hypothermic cardioplegic arrest [23]. Tofukuji and colleagues also have demonstrated that the agent (40 mg/kg), administered intravenously before reperfusion, reduced neutrophil infiltration and endothelial injury in the coronary and cerebral microvasculature after cardiopulmonary bypass, whereas it influenced neither perfusion nor function of the myocardium and brain [24].

In the present study, we found that intermittent antegrade infusion of warm blood cardioplegia supplemented with Sialyl LewisX analogue preserved left ventricular systolic (dp/dt) and diastolic (-dp/dt) function and endothelial function better than the control group during reperfusion. Because MPO is found almost exclusively within neutrophils, the increased MPO activity in a certain tissue is regarded as an index of neutrophil infiltration [16]. Increased infiltration and accumulation of activated neutrophils into myocardial tissue during ischemia-reperfusion is considered to be an important mechanism that con-
tributes to the reperfusion injury. We, therefore, measured myeloperoxidase activity as a marker for neutrophil accumulation. Histological evidence of neutrophil infiltration has been reported to be correlated with the content of myocardial MPO activity [16]. The lower MPO activity in the myocardial tissue found in the present study, therefore, indicated the inhibition of neutrophil-mediated reperfusion injury by Sialyl Lewis\textsuperscript{x} analogue.

The inhibitory effect of the agent on reperfusion injury may be mainly due to the preservation of endothelial function, because it has been shown that endothelial dysfunction occurs within 2.5-5 min of reperfusion [25], and leads to the upregulation of P-selectin within 10-20 min [19]. Mullane et al. [4] have demonstrated in an ischemia and reperfusion model that the adhesion of neutrophils to the endothelium occurred within 30 min of reperfusion, whereas the transition of neutrophils to the myocardium was observed after 1 hour of reperfusion. It has been suggested that a significant degree of myocardial tissue injury may be amplified later by neutrophils adhering to myocytes [3], and the damage assessed by either elevated plasma CK activities or increasing mass of necrotic tissue was evident after longer reperfusion periods (3-4.5 h) [25]. Both oxygen-free radical production [26] and release of proteolytic enzymes from neutrophil granules [27] have been shown to be mediated by CD11b/CD18-dependent adhesion of neutrophils. These relationships are supported by the clinical observation of a temporal relationship between CD11b/CD18 expression and a rise in plasma levels of elastase [28]. Because the integrins mediate the firm adhesion at a later stage, our sampling point might have been too early to assess the firm adhesion and infiltration of neutrophils. We believe that this may explain why no differences were found in the ELAS, TBARS, and CKMB levels between the groups.

It has been proven that effects of Sialyl Lewis\textsuperscript{x} were dose-dependent [10,29]. In our experiments, although we used considerably lower dose (3.75-4.3 mg/kg) than other global ischemia reperfusion models (40 mg/kg or 40 mg/l) [23,24], our results demonstrated similar effects of the agent. In the previous studies, Sialyl Lewis\textsuperscript{x} was given systemically in a single-dose either before ischemia or immediately before reperfusion. According to David and coworkers, endothelial P-selectin expression, that recruit the first wave of neutrophils, was already induced during hypoxia [30]. Moreover, the half-life of Sialyl Lewis\textsuperscript{x} in blood is considerably short (15-30 min) [10,11,31]. In the present study, the administration of the drug was instituted at the onset of ischemia. And the coronary vasculature was replenished with the drug every 20 min during ischemia. We believe that this manner of administration was associated with a similar inhibitory effects on reperfusion injury despite of the lower-dose.

A major limitation of the present study is the limited duration of reperfusion. It would be necessary to study the effects of Sialyl Lewis\textsuperscript{x} analogue on reperfusion injury for a longer period during reperfusion, because there is a possibility that the drug merely delayed the onset of myocardial injury. Thus, the results might have been different if we had investigated its effect at later period of reperfusion.

Another important issue regarding the clinical application of this therapy is whether the agent has adverse side effect in human. To elucidate this, further investigations using primates are required.

In summary, the results suggested that selectin-mediated endothelial-leukocyte interactions may play an important role in the myocardial ischemia and reperfusion injury that is frequently observed in clinical cardiac operations. Warm blood cardioplegia supplemented with Sialyl Lewis\textsuperscript{x} analogue preserved left ventricular function, and endothelial function by an inhibition of selectin-mediated endothelial-leukocyte interactions during early reperfusion period.

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EFFECTS OF SIALYL LEWIS\(^x\) DURING CARDIOPLEGIA


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