CARDIOTHORACIC TRANSPLANTATION

NITROGLYCERIN AS A NITRIC OXIDE DONOR ACCELERATES LIPID PEROXIDATION BUT PRESERVES VENTRICULAR FUNCTION IN A CANINE MODEL OF ORTHOTOPIC HEART TRANSPLANTATION

Yoshihisa Tanoue, MD
Shigeki Morita, MD
Yoshie Ochiai, MD
Naho Haraguchi, MD
Ryuji Tominaga, MD
Yoshito Kawachi, MD
Hisataka Yasui, MD

Background: Nitric oxide has cardioprotective effects through several mechanisms. However, nitric oxide may have deleterious effects in the presence of superoxide because it is converted to peroxynitrite, which then initiates lipid peroxidation. Using a canine model of orthotopic heart transplantation, we examined whether adding an organic nitric oxide donor, nitroglycerin, to preservation solution elicits lipid peroxidation after reperfusion and causes deleterious effects on coronary endothelial function and left ventricular function. **Methods and results:** The donor heart was preserved for 24 hours in cold University of Wisconsin solution with nitroglycerin (0.1 mg/mL) supplementation (group NTG, n = 8) or in standard University of Wisconsin solution (group C, n = 8). After reperfusion, changes of coronary resistance were measured during the infusion of acetylcholine (0.1 mg/min) and of sodium nitroprusside (1 mg/min), and percent coronary relaxation was calculated. Left ventricular function was evaluated by pressure-volume relations with the use of a conductance catheter, thereby deriving the slopes of end-systolic pressure-volume relation, stroke work–end-diastolic volume relation, and maximum rate of change of left ventricular pressure–end-diastolic volume relation. Serum lipid peroxide level was measured. Percent coronary relaxation was similar for the 2 groups. The slopes of end-systolic pressure-volume relation, stroke work–end-diastolic volume relation, and maximum rate of change of left ventricular pressure–end-diastolic volume relation in group NTG were significantly higher than those in group C. On the other side, serum lipid peroxide level in group NTG was significantly higher than that in group C. **Conclusions:** Nitroglycerin may have detrimental effects evidenced by the increase in lipid peroxidation, which implied peroxynitrite formation. However, the overall effect of nitroglycerin was cardioprotective. Although the exact mechanism is yet to be clarified, the superb cardioprotective effect of nitroglycerin overwhelms the exaggeration of lipid peroxidation. (J Thorac Cardiovasc Surg 1999;118:547-56)

Nitric oxide (NO) has cardioprotective effects through several mechanisms, such as potent vasodilator actions,¹ antiplatelet activity,² and inhibition

of both neutrophil aggregation and adhesion.^{3,4} After myocardial ischemia and reperfusion, release of NO from coronary endothelium decreases.^{5,6} Thus an NO

From The Department of Cardiovascular Surgery, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

Presented in part at the Sixty-ninth Scientific Sessions of the American Heart Association, New Orleans, Louisiana, November 10-13, 1996, and published in abstract form (Circulation 1996; 94[Suppl]:I53).

Received for publication Nov 23, 1998; revisions requested Feb 19, 1999; revisions received May 21, 1999; accepted for publication May 26, 1999.

Address for reprints: Shigeki Morita, MD, Department of Cardiovascular Surgery, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan (E-mail: morita@heart.med.kyushu-u.ac.jp).

Copyright © 1999 by Mosby, Inc.

0022-5223/99 \$8.00 + 0 12/1/100277

donor given during the course of ischemia and reperfusion might preserve coronary endothelial function and decrease myocardial injury. However, in the presence of superoxide, NO rapidly reacts with superoxide to form peroxynitrite, which is a highly reactive species that can induce lipid peroxidation and cause coronary endothelial damage and myocardial injury.⁷ Therefore NO during ischemia and reperfusion would have both cardioprotective and harmful effects. Nitroglycerin (NTG) is an organic NO donor and NTG supplementation in preservation solution has been reported to enhance cardiac^{8,9} and pulmonary preservation.¹⁰ However, NTG might cause the formation of peroxynitrite and accelerate lipid peroxidation after reperfusion.

The purpose of this study was to describe the *in vivo* efficacy of adding NTG in preservation solution. For this purpose, coronary endothelial function, left ventricular (LV) function, and metabolites of NO and lipid peroxide levels were measured during the course of ischemia and reperfusion with the use of the clinically relevant canine model of orthotopic heart transplantation.

Materials and methods

Donor preparation. Sixteen adult mongrel dogs were divided randomly into 2 groups: the NTG-supplemented group (group NTG, *n* = 8) and the control group (group C, *n* = 8). All donor dogs were anesthetized with sodium pentobarbital (30 mg/kg intravenously) followed by the intramuscular administration of atropine sulfate (0.04 mg/kg). The dogs were intubated, their lungs mechanically ventilated, and then placed in a supine position. Pancuronium bromide (0.1 mg/kg) was administered intravenously for muscle relaxation. A continuous infusion of sodium pentobarbital was used to maintain anesthesia. An antibiotic, flomoxef (25 mg/kg intravenously), was administered to prevent infection. Systemic blood pressure was continuously monitored.

After a median sternotomy, heparin sodium (500 U/kg intravenously) was administered systemically, and the heart and blood of the donor dog were removed as described before.^{11,12} The heart was arrested by the infusion of cold crystalloid cardioplegic solution (15 mL/kg; K⁺ 20 mmol/L, Na⁺ 87 mmol/L, Ca⁺⁺ 0.1 mmol/L, Cl⁻ 97 mmol/L, HCO₃⁻ 10 mmol/L, glucose 25 gm/L).¹² The heart was then flushed with cold University of Wisconsin solution (15 mL/kg; ViaSpan, Du Pont Pharmaceuticals, Wilmington, Del) infused at a constant rate over a 2-minute period with a rotating pump (Mera Blood Pump HCP-100, Senko Medical Instrument, Tokyo, Japan). During the infusion of University of Wisconsin solution, aortic root pressure was measured. Eight donor hearts in group NTG were flushed and preserved in University of Wisconsin solution with NTG (0.1 mg/mL; Du Pont Pharmaceuticals) supplementation, whereas 8 donor hearts in group C, flushed and preserved in standard University of

Wisconsin solution, served as a control. The temperature of solution was maintained at 4°C. The hearts were placed in a plastic bag containing the cold University of Wisconsin solution and were preserved at 1°C for 24 hours.

Recipient preparation. After 24 hours, the hearts were transplanted into 16 weight-matched recipient dogs by a standard technique of orthotopic heart transplantation. All recipient dogs were anesthetized and pretreated in the same manner as the donor dogs and received methylprednisolone (500 mg intravenously) and indomethacin (50 mg by rectum) after induction for the purpose of stabilizing the cardiopulmonary bypass preparation.

All recipients were placed in a supine position. After a median sternotomy and heparinization (500 U/kg intravenously), cardiopulmonary bypass with moderate hypothermia was instituted with a heart-lung machine consisting of a centrifugal pump (Bio-Pump BP-80; Medtronic Bio-Medicus, Eden Prairie, Minn) and a membrane oxygenator (D705 Midiflo System; Dideco, Mirandola, Italy), which was primed with blood from the donor dog. After the ascending aorta had been crossclamped, the recipient heart was removed, and the donor heart was transplanted. The donor heart was topically cooled with iced slush and was flushed twice with cold crystalloid cardioplegic solution (5 mL/kg) during the procedure. After reperfusion, the heart was electrically defibrillated, if necessary. Fifteen minutes after reperfusion, calcium chloride was given to correct the serum calcium level, and 30 minutes after reperfusion, ventricular pacing was instituted at a rate of 150 beats/min by the external pacer (Chronocor VI, model 5012, Teletronics Pacing Systems, Englewood, Colo).

The heart rate was maintained at 150 beats/min by pacing the right ventricle during the assessment period. All signals (electrocardiogram, pressures, flows, and volume) were continuously monitored on a multichannel oscillograph (Polygraph 360 system, NEC Sanei, Tokyo, Japan) and on-line digitized at 200 Hz with an analog-to-digital converter (MacLab System, ADInstruments, Ltd, Dunedin North, New Zealand) and recorded on a digital computer (Macintosh Quadra 700, Apple Computer, Inc, Cupertino, Calif). After the measurements, all dogs were killed by means of an overdose of intravenous sodium pentobarbital and potassium chloride. Each dog's heart was removed from the chest, and the right and left ventricles (the free wall and the septum) were weighed. LV free wall specimens were taken for the measurement of myocardial water content.

Data analysis

Coronary endothelial function. Up to 150 minutes after reperfusion, coronary endothelial function was assessed during cardiopulmonary bypass with the left ventricle vented. At 15, 30, 60, and 150 minutes after reperfusion, coronary vascular resistance was measured without inotropic or vasodilatory agents. Coronary vascular resistance was calculated as the mean aortic root pressure divided by the mean coronary flow. During 70 to 150 minutes after reperfusion, endothelium-dependent and -independent coronary relaxations were induced by direct infusions of acetylcholine and sodium

Fig 1. A, Cardiopulmonary bypass for the assessment of coronary endothelial function. The coronary vessels were isolated from the systemic circuit by crossclamping the ascending aorta and were perfused with oxygenated blood through the side arm of the arterial line. Acetylcholine and sodium nitroprusside were infused from this side arm for the assessment of coronary endothelial function. *ACh*, Acetylcholine; *SNP*, sodium nitroprusside; *RV*, right ventricular; *LV*, left ventricular. **B,** Right heart bypass for the assessment of left ventricular function. To measure the left ventricular pressure–volume loop, a catheter-tip micromanometer and conductance catheter were inserted into the left ventricle. Right heart bypass was instituted to control the left ventricular venous return and to completely decompress the right ventricle, thereby eliminating parallel conductance variation.

nitroprusside, respectively, as described previously.¹³ As shown in Fig 1, A, the coronary vessels were isolated from the systemic circuit by crossclamping the ascending aorta and were perfused with oxygenated blood through a 14-gauge cannula connected to the side arm of the arterial line. To drain the entire coronary venous return for the flow measurement, a cannula was placed in the right ventricle through the right atrium, and the tapes around the superior and inferior venae cavae and the main pulmonary artery were snared. Coronary flow was measured by an in-line electromagnetic flow probe connected with a flowmeter (model MFV-3200, Nihon Kohden, Tokyo, Japan). The coronary pressure and flow were allowed to self-regulate, and they were recorded at baseline and during the infusion of 3, 10, 30, and 100 μ g of acetylcholine for 60 seconds. Coronary pressure and flow were then allowed to return to baseline, and they were recorded after 1000 μ g of sodium nitroprusside for 60 seconds. Percent coronary relaxation was calculated as (baseline resistance – minimal resistance)/baseline resistance $\times$ 100 (%).

Conventional hemodynamic variables. All recipients were disconnected from cardiopulmonary bypass 150 minutes after reperfusion without inotropic or vasodilatory agents, and conventional hemodynamic variables (heart rate, left atrial pressure, mean aortic pressure, and aortic flow) were obtained. The left atrial pressure was maintained at 10 mm Hg during the assessment period.

LV function. For 150 to 180 minutes after reperfusion, LV function was assessed during right heart bypass without inotropic or vasodilatory agents. A right heart bypass prepa-

ration was established as shown in Fig 1, B.^{11,12} The tapes around the superior and inferior venae cavae were snared to direct systemic venous blood return into a reservoir. An arterial cannula was inserted into the main pulmonary artery, and the blood oxygenated with a membrane oxygenator was pumped back to the main pulmonary artery. The entire coronary venous return was drained for the flow measurement and the oximetry. To measure the LV volume, a 7F 12-electrode conductance catheter (Sentron B.V., Roden, The Netherlands) was inserted into the left ventricle through the apex. The catheter was attached to a signal generator/processor (Sigma 5, Leycom, Oegstgeest, The Netherlands).¹⁴ A catheter-tip micromanometer (model MPC-500, Millar Instruments, Inc, Houston, Tex) was inserted into the left ventricle, and LV pressure–volume loops were then constructed. A 14-mm ultrasonic flow probe connected with the flowmeter (model T208, Transonic Systems, Inc, New York, NY) was positioned around the ascending aorta for the measurement of aortic flow to calibrate the volume signal of the conductance catheter. Right heart bypass was instituted to control the LV venous return and to decompress the right ventricle completely, thereby eliminating parallel conductance variation. The parallel conductance volume was calculated by transiently altering blood conductivity by the injection of hypertonic saline solution (5 mL of 10% NaCl).¹⁴

Multiple LV pressure–volume loops were obtained during transient preload reduction by reducing bypass flow. The digitized data were analyzed by computer algorithms using a C-language-type program developed in our laboratory with an

Fig 2. The changes of coronary vascular resistance at 15, 30, 60, and 150 minutes after reperfusion in group NTG and group C. Two-factor analysis of variance with repeated measures on 1 factor showed that the changes of coronary vascular resistance were marginally different between the 2 groups ($P = .08$). Data are presented as mean $\pm$ standard deviation.

Intel 486-based personal computer (Vision, IBM Japan, Tokyo, Japan). LV contractility was evaluated by the end-systolic pressure-volume (P_{es} - V_{es}) relation,¹⁵ the stroke work-end-diastolic volume (SW - V_{ed}) relation,¹⁶ and the maximum rate of change of LV pressure-end-diastolic volume (dP/dt_{max} - V_{ed}) relation.¹⁷ The P_{es} - V_{es} relation was fitted by linear regression to obtain the slope (E_{es}) and the volume intercept ($V_{0,es}$), and the volume associated with P_{es} of 100 mm Hg ($V_{100,es}$) was calculated. SW calculated as the area inside the pressure-volume loop was plotted against V_{ed} to obtain the slope (M_{SW}) and the volume intercept ($V_{0,SW}$) of the SW - V_{ed} relation. The end-diastolic point was defined as the point of the upstroke of the first derivative of the LV pressure (dP/dt). The position of the SW - V_{ed} relation in the operating range was calculated by determining V_{ed} associated with SW of 500 mm Hg $\cdot$ mL ($V_{500,SW}$). In the same way, the slope (dE/dt_{max}) and the volume intercept ($V_{0,dP/dt}$) of the dP/dt_{max} - V_{ed} relation were obtained, and V_{ed} associated with a dP/dt_{max} of 1000 mm Hg/sec ($V_{1000,dP/dt}$) was calculated. In addition, LV stiffness (β) was determined by fitting end-diastolic pressure-volume points to the exponential relation as described previously.¹⁸

Myocardial energetics. During 150 to 180 minutes after reperfusion, multiple steady-state pressure-volume loops, coronary flow, and arteriovenous oxygen difference data were obtained at various preload volumes for the assessment of myocardial energetics. Myocardial energetics was assessed by the analysis of the relation between LV myocardial oxygen consumption and systolic pressure-volume area as described by Suga.¹⁵

Creatine kinase MB isoenzyme, lipid peroxide, and metabolites of NO. Serum creatine kinase-MB isoenzyme (CK-MB) level, serum lipid peroxide level, and the total plasma concentrations of nitrite and nitrate were measured before reperfusion and at 15, 30, 60, and 150 minutes after reperfusion.

Fig 3. Percent endothelium-dependent coronary relaxation induced by acetylcholine and percent endothelium-independent coronary relaxation induced by sodium nitroprusside in group NTG and group C. The 2 groups were similar with respect to percent endothelium-dependent coronary relaxation induced by acetylcholine and percent endothelium-independent coronary relaxation induced by sodium nitroprusside. *ACh*, Acetylcholine; *SNP*, sodium nitroprusside. Data are presented as mean $\pm$ standard deviation.

Blood samples were obtained from the coronary sinus. Serum lipid peroxide level was measured by means of a methylene blue derivative method.¹⁹ The total plasma concentrations of nitrite and nitrate were measured by converting into NO with *Aspergillus* nitrate reductase and hydrochloric acid.²⁰ The amount of produced NO was then measured by an NO analyzer (model 270B, Sievers Instruments, Inc, Boulder, Colo).

Myocardial water content. LV free wall specimens taken after experiments were weighed just after collection (wet weight) and after 24 hours' desiccation (dry weight). The myocardial water content was calculated by the formula:

$$\text{Myocardial water content} = (\text{Wet weight} - \text{Dry weight}) / \text{Wet weight} \times 100 (\%).$$

Statistical analysis. Results are presented as mean $\pm$ standard deviation. The Student unpaired t test was used for the variables determined once during the experiment to examine the difference between the 2 groups. Two-factor analysis of variance with repeated measures on 1 factor²¹ was used for the variables measured in series. This analysis clarified whether each of (1) repeated measures factor and (2) non-repeated measures factor was significantly different among or between the levels and also whether (3) interaction between the 2 factors was significant. Our major interest in using this analysis (2-factor analysis of variance with repeated measures on 1 factor) was to see whether the variables were statistically different between group NTG and group C.

Animal care. All animals 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" pre-

pared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). This experiment was reviewed by the Committee of the Ethics on Animal Experiment in the Faculty of Medicine, Kyushu University, and carried out under the control of the Guidelines for Animal Experimentation in the Faculty of Medicine, Kyushu University and The Law (No. 105) and Notification (No. 6) of the Government.

Results

All the dogs in both groups were successfully disconnected from cardiopulmonary bypass. In group C all dogs required ventricular pacing, whereas in group NTG, 5 of 8 dogs were weaned from cardiopulmonary bypass in sinus rhythm. No significant difference was observed between the 2 groups in regard to donor weight (group NTG, 16.1 ± 1.7 kg; group C, 15.0 ± 1.5 kg; $P = .19$) and aortic root pressure during the infusion of University of Wisconsin solution (group NTG, 42.3 ± 5.9 mm Hg; group C, 44.4 ± 3.0 mm Hg; $P = .4$). There was no difference in aortic flow measured at a left atrial pressure of 7 mm Hg for the assessment of cardiac function of pretransplant donor hearts (group NTG, 1119 ± 196 mL/min; group C, 1102 ± 298 mL/min; $P = .9$). The 2 groups were similar with respect to recipient weight (group NTG, 16.4 ± 1.4 kg; group C, 15.3 ± 1.4 kg; $P = .16$), total ischemic time (group NTG, 24.5 ± 0.8 hours; group C, 24.6 ± 0.8 hours; $P = .98$), and implantation time (group NTG, 74.1 ± 7.8 minutes; group C, 71.9 ± 10.8 minutes; $P = .6$).

Coronary endothelial function. The changes of coronary vascular resistance 15, 30, 60, and 150 minutes after reperfusion are shown in Fig 2. Two-factor analysis of variance with repeated measures on 1 factor showed that the changes of coronary vascular resistance were marginally different between the 2 groups ($P = .08$), but neither coronary vascular resistance against time nor the interaction term was significant ($P = .9$, $P = .10$, respectively). These statistical analyses indicate that coronary vascular resistance tended to be lower in the NTG group than in the control group, but the changes of coronary vascular resistance could not be statistically proved to be dependent on elapsing time after reperfusion. As shown in Fig 3, the 2 groups were similar with respect to percent endothelium-dependent coronary relaxation induced by acetylcholine and percent endothelium-independent coronary relaxation induced by sodium nitroprusside.

Conventional hemodynamic variables. Conventional hemodynamic variables at 150 minutes after reperfusion are provided in Table I. Mean aortic pres-

Table I. Comparisons of the hemodynamics between group NTG and group C after reperfusion

	HR (beats/min)	LAP (mm Hg)	mAOP (mm Hg)	AOF (mL/min)
Group NTG	150 ± 4	10.3 ± 0.8	91.0 ± 24.1	671 ± 150
Group C	151 ± 3	10.3 ± 0.9	68.1 ± 13.1	420 ± 172
P value	.6	.9	.03	.008

Data are presented as mean $\pm$ standard deviation. Hemodynamics was measured without inotropic and vasodilatory agents. HR, Heart rate; LAP, left atrial pressure; mAOP, mean aortic pressure; AOF, aortic flow.

sure and aortic flow in group NTG were significantly higher than those in group C.

LV function. Parameters of systolic ventricular function are provided in Table II. Indices of systolic ventricular function, E_{es} , M_{SW} , and dE/dt_{max} , of group NTG were all significantly higher than those of group C. The differences of $V_{0,es}$ and $V_{0,SW}$ between the 2 groups were significant. All 3 relations in group NTG were shifted significantly toward the left in the operating range compared with those of group C, manifested by the differences in $V_{100,es}$, $V_{500,SW}$, and $V_{1000,dP/dt}$. The parameters of contractility of 10 normal adult mongrel dogs measured during right heart bypass without inotropic or vasodilatory agents are shown in Table II to appreciate the degree of dysfunction after transplantation. The 2 groups were similar with respect to parameter of diastolic ventricular function, the degree of curvature that reflected end-diastolic chamber stiffness, β (group NTG, 0.108 ± 0.040 ; group C, 0.108 ± 0.047 ; $P = .99$). Chamber stiffness, β , of 10 normal adult mongrel dogs was 0.084 ± 0.025 .

Myocardial energetics. The 2 groups were similar with respect to the slope of the myocardial oxygen consumption–systolic pressure–volume area relation (group NTG, $1.69 \pm 0.44 \times 10^{-5}$; group C, $1.18 \pm 0.76 \times 10^{-5}$; $P = .12$), and the intercept (group NTG, $1.34 \pm 0.50 \times 10^{-2}$; group C, $1.36 \pm 0.77 \times 10^{-2}$; $P = .95$).

CK-MB isoenzyme, lipid peroxide, and metabolites of NO. The changes of serum CK-MB level, serum lipid peroxide level, and the total plasma concentrations of nitrite and nitrate before reperfusion and 15, 30, 60, and 150 minutes after reperfusion in group NTG and group C are shown in Fig 4. No significant difference was observed between the 2 groups in regard to serum CK-MB level, serum lipid peroxide level, and the total plasma concentrations of nitrite and nitrate before reperfusion. Serum CK-MB levels in group NTG tended to be higher than those in group C, although the differences did not reach statistical significance. Two-factor analysis of variance with repeated

Table II. Comparisons of the P_{es} - V_{es} , SW - V_{ed} , and dP/dt_{max} - V_{ed} relations between group NTG and group C after reperfusion

	P_{es} - V_{es} relation		
	E_{es} (mm Hg/mL)	$V_{0,es}$ (mL)	$V_{100,es}$ (mL)
Group NTG	9.3 ± 3.4	-2.4 ± 3.5	9.7 ± 2.9
Group C	6.1 ± 2.2	8.3 ± 11.1	26.5 ± 10.5
P value	.04	.02	.0006
Normal*	9.1 ± 2.6	1.0 ± 5.2	13.4 ± 5.3
	SW - V_{ed} relation		
	M_{SW} (mm Hg)	$V_{0,SW}$ (mL)	$V_{500,SW}$ (mL)
Group NTG	58.3 ± 17.5	7.8 ± 3.7	17.0 ± 4.0
Group C	36.4 ± 10.0	19.2 ± 11.5	33.8 ± 11.8
P value	.008	.02	.002
Normal*	88.7 ± 18.5	10.4 ± 5.1	16.3 ± 5.7
	dP/dt_{max} - V_{ed} relation		
	dE/dt_{max} (mm Hg/[sec · mL])	$V_{0,dP/dt}$ (mL)	$V_{1000,dP/dt}$ (mL)
Group NTG	79.4 ± 13.8	-3.0 ± 5.0	9.9 ± 3.5
Group C	54.9 ± 28.5	3.4 ± 20.3	26.1 ± 11.2
P value	.04	.4	.002
Normal*	80.9 ± 10.8	-2.0 ± 4.6	11.9 ± 2.6

P_{es} , Left ventricular end-systolic pressure; V_{es} , end-systolic volume; E_{es} , slope of P_{es} - V_{es} relation; $V_{0,es}$, volume axis intercept of P_{es} - V_{es} relation; $V_{100,es}$, V_{es} associated with P_{es} of 100 mm Hg; SW , left ventricular stroke work; V_{ed} , end-diastolic volume; M_{SW} , slope of SW - V_{ed} relation; $V_{0,SW}$, volume intercept of SW - V_{ed} relation; $V_{500,SW}$, V_{ed} associated with stroke work of 500 mL · mm Hg; dP/dt_{max} , maximum rate of change of left ventricular pressure; dE/dt_{max} , slope of dP/dt_{max} - V_{ed} relation; $V_{0,dP/dt}$, volume intercept of dP/dt_{max} - V_{ed} relation; $V_{1000,dP/dt}$, V_{ed} associated with dP/dt_{max} of 1000 mm Hg/sec.

*Values of 10 normal adult mongrel dogs measured during right heart bypass without inotropic or vasodilatory agents. Data are presented as mean ± standard deviation. Left ventricular systolic function was measured without inotropic and vasodilatory agents.

measures on 1 factor showed that serum lipid peroxide levels after reperfusion in group NTG were significantly higher than those in group C ($P = .02$) and that the total plasma concentrations of nitrite and nitrate after reperfusion in group NTG were significantly higher than those in group C ($P = .001$).

Myocardial water content. No significant difference in the myocardial water content was observed between group NTG and group C: 76.6% ± 3.7% and 78.8% ± 1.1% ($P = .12$), respectively.

Discussion

Myocardial ischemia followed by reperfusion results in coronary endothelial dysfunction and myocardial injury. NO released by endothelial cells has many physiologic functions and plays an important role in modulating ischemia-reperfusion injury. Several studies have shown NO to have cardioprotective effects,^{2,3,22,23} but other data suggest that NO can exaggerate ischemia-reperfusion injury by reacting with superoxide to form peroxynitrite.^{7,24} Adding NTG, an organic NO donor, to cardiac preservation solution would produce both cardioprotective and deleterious effects. Pinsky and

colleagues,⁸ using a heterotopic rat transplantation model, studied the effects of NO donors as additives to cardiac preservation solution. They reported that the addition of NTG to preservation solution enhanced graft survival. Oz and associates⁹ reported the superiority of NTG-containing preservation solution in rat and baboon cardiac transplantation models. On the other hand, some studies have reported the harmful effects of NO. Finkel and colleagues²⁵ reported the negative inotropic effects of NO in isolated hamster papillary muscles. Matheis and associates,²⁴ using a piglet hypoxic model, showed that NO was involved in myocardial reoxygenation injury. Brady and colleagues²⁶ reported that NO reduced cardiac contractility in guinea pig cardiac ventricular myocytes. The effects of NO on cardiac tissue are still controversial, especially during the course of ischemia and reperfusion.

As we have hypothesized, NTG administration caused an increase in serum lipid peroxide levels after reperfusion, which indicated acceleration of lipid peroxidation. To the best of our knowledge, this is the first study to demonstrate the elevation of serum lipid peroxide level by the administration of NTG in a clinical-

Fig 4. Serum CK-MB levels (A), serum lipid peroxide levels (B), and the total plasma concentrations of nitrite and nitrate (C) before reperfusion and at 15, 30, 60, and 150 minutes after reperfusion in group NTG and group C. Serum CK-MB levels in group NTG tended to be higher than those in group C, although the differences did not reach statistical significance. Two-factor analysis of variance with repeated measures on 1 factor showed that serum lipid peroxide levels after reperfusion in group NTG were significantly higher than those in group C ($P = .02$) and that the total plasma concentrations of nitrite and nitrate after reperfusion in group NTG were significantly higher than those in group C ($P = .001$). CK-MB, Creatine kinase-MB; LPO, lipid peroxide; NO₂+NO₃, total plasma concentrations of nitrite and nitrate. Data are presented as mean $\pm$ standard deviation.

ly relevant canine model of orthotopic heart transplantation. Although the difference did not reach statistical significance, serum CK-MB levels after reperfusion of NTG-treated dogs also tended to be higher. Since lipid peroxidation is a free radical-induced chain reaction involving polyunsaturated fatty acids in cell membranes,²⁷ these findings evidenced the harmful effect of NO donated by NTG. These potentially deleterious

effects might be caused by peroxynitrite, which was produced from NO in the presence of superoxide.⁷

No difference in coronary endothelial function was detected between the 2 groups in this study. Our pilot studies showed that percent coronary relaxations during the infusion of both 100 μ g of acetylcholine and 1000 μ g of sodium nitroprusside in normal dogs were more than 75%. Therefore coronary endothelial func-

tion both of dogs receiving NTG and of those not receiving NTG was similarly impaired. On the other hand, conventional hemodynamic variables (mean aortic pressure and aortic flow; Table I) of dogs treated with NTG were superior to those of dogs without NTG treatment. In addition, the load-independent parameters of systolic ventricular function, E_{es} , M_{SW} , and dE/dt_{max} , of dogs treated with NTG were superior to those of dogs without NTG treatment (Table II). Although there was no difference in LV end-diastolic chamber stiffness between the 2 groups, LV pressure-volume loops of dogs treated with NTG shifted significantly toward the left compared with those of dogs without NTG treatment. These results strongly indicated that contractility of NTG-treated dogs was well preserved despite the exaggeration of lipid peroxidation. Since we did not administer any inotropic agents for the weaning from cardiopulmonary bypass, and since the implanted hearts were completely denervated, it may be safely assumed that the improved ventricular function directly reflected preserved intrinsic contractility of hearts treated with NTG.

Several mechanisms of the cardioprotective effect of NO have been postulated. One of the earliest observed abnormalities in ischemia-reperfusion injury is an endothelial dysfunction manifested by a loss of NO-dependent vasodilation.^{5,6} Thus the administration of an NO donor during the course of ischemia and reperfusion is a reasonable strategy for the treatment of ischemia-reperfusion injury. The inhibition of neutrophil aggregation and adhesion by NO also could play a greater role because the postischemic blood flow defects, so-called "no-reflow" phenomenon, after reperfusion is mediated by neutrophil embolization of the resistance vessels.²⁸ In addition, the antiplatelet activity of NO also could contribute to the improvement of ventricular function by preventing coronary thrombosis. Besides the cardioprotective effect of NO, NO might have a direct effect on myocytes to improve cardiac contractile function. Kojda and colleagues²⁹ reported that organic nitrates such as NTG stimulated cyclic adenosine monophosphate-dependent protein kinase, thereby improving contractile response of rat ventricular myocytes. These cardioprotective effects and the enhancement of contractility with NO might have counteracted the NO-induced exaggeration of lipid peroxidation, which resulted in superior ventricular function of the NTG-treated hearts. Our finding implied that NTG has a cardioprotective effect, despite evidence of increased lipid peroxidation. Our preliminary experiment using an isolated rabbit heart preparation showed similar paradoxical findings with L-arginine.

The result indicated that these paradoxical findings are related to NO itself and not limited to NTG. Since L-arginine is known to suppress neutrophil adhesion to the endothelium, the mechanism of cardioprotective effects of NTG may relate to inhibition of neutrophil activation. Further study is warranted to investigate the exact mechanism and interaction of the cardioprotective and free radical-producing effects of NO produced from NTG, and NTG itself.

Our results showed higher levels of nitrite and nitrate in the NTG-treated dogs after reperfusion. Persistently higher level of nitrite and nitrate was probably due to the longer half-life of nitrate (3.8 hours).²¹ NTG is an organic NO donor; that is, the conversion from NTG to NO requires enzymatic activity.³⁰ It was very likely that NTG was not converted to NO until the time of reperfusion, because the enzymatic activity responsible for NO production must have been suppressed during the cold preservation period. This presumption was partly supported by the finding that there was no difference in aortic root pressure during the infusion of University of Wisconsin solution with or without NTG at an infusion temperature of 4°C. The feature of the enzyme-dependent NO production may be the advantage of NTG over spontaneous NO donors, such as sodium nitroprusside.³⁰ Spontaneous NO donors may release NO during the cold preservation period. Since the half-life of NO is extremely short, no NO would be available at the time of reperfusion if spontaneous NO donors were used as additives to preservation solution. NTG contained in University of Wisconsin solution may accumulate in coronary vessel walls and cardiac myocytes during preservation. Accumulated NTG may enzymatically convert to NO immediately after reperfusion. High-dose NO immediately after reperfusion would have a cardioprotective effect. We therefore assume that organic NO donors should be used as supplements to preservation solution. The concentration of NTG in the University of Wisconsin solution used in our study was extremely high, which would cause severe hypotension when given systemically or in terminal warm blood cardioplegic solution. Thus adding NTG in preservation solution was the only way to give high-dose NTG.

In clinical practice, University of Wisconsin solution has been introduced for heart preservation and has been used widely. The ischemic time, however, is still limited to 6 to 8 hours with University of Wisconsin solution. Judging from our result in a clinically relevant experimental model, we may be able to extend preservation time by adding NTG to University of Wisconsin solution. Before the clinical application, however, fur-

ther study is warranted to investigate the exact mechanism of the cardioprotective effect of NTG and to develop a method to prevent the deleterious effect of NTG.

The limitation of our experimental model was that the assessment period was limited to 180 minutes after reperfusion, because we were obliged to use right heart bypass with a membrane oxygenator for functional measurement. To examine the long-term effect of NTG, we measured conventional hemodynamic parameters without using right heart bypass. In this preliminary study, the hearts of 3 dogs preserved with University of Wisconsin solution containing NTG were able to maintain adequate cardiac output up to 8 hours after reperfusion. At the left atrial pressure of 10 mm Hg, cardiac output was 531 ± 196 mL/min without inotropic agents and 1035 ± 468 mL/min with dobutamine infusion at a rate of $5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. The cardiac preservation period of 24 hours was selected because our preliminary reports showed no difference between the 2 groups after 6 or 12 hours of preservation in University of Wisconsin solution. Right ventricular pacing was performed in all animals to fix the heart rate at 150 beats/min during the assessment period because of the parameters of contractility affected by the heart rate. Although the 2 groups were similar with respect to end-diastolic chamber stiffness, right ventricular pacing would influence the measurement of end-diastolic pressure–volume relations.

In conclusion, NTG may have detrimental effects evidenced by the increase in lipid peroxidation. However, the overall effect of NTG was cardioprotective. Although the exact mechanism is yet to be clarified, the superb cardioprotective effect of NTG overwhelms the exaggeration of lipid peroxidation.

This study was prepared in consultation with Yasuhiko Harasawa, MD, who assisted in the statistical analyses.

REFERENCES

1. Furchgott RF. Role of endothelium in responses of vascular smooth muscle. *Circ Res* 1983;53:557-73.
2. Pohl U, Busse R. EDRF increases cyclic GMP in platelets during passage through the coronary vascular bed. *Circ Res* 1989;65:1798-803.
3. Lefer DJ, Nakanishi K, Johnston WE, Vinten-Johansen J. Antineutrophil and myocardial protecting actions of a novel nitric oxide donor after acute myocardial ischemia and reperfusion in dogs. *Circulation* 1993;88[part 1]:2337-50.
4. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci U S A* 1991;88:4651-5.
5. Tsao PS, Aoki N, Lefer DJ, Johnson G III, Lefer AM. Time course of endothelial dysfunction and myocardial injury during myocardial ischemia and reperfusion in the cat. *Circulation* 1990;82:1402-12.
6. Van Benthuyzen KM, McMurtry IF, Horwitz LD. Reperfusion after acute coronary occlusion in dogs impairs endothelial-dependent relaxation to acetylcholine and augments contractile reactivity in vitro. *J Clin Invest* 1987;79:265-74.
7. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 1990;87:1620-4.
8. Pinsky DJ, Oz MC, Koga S, Taha Z, Broekman MJ, Marcuc AJ, et al. Cardiac preservation is enhanced in a heterotopic rat transplant model by supplementing the nitric oxide pathway. *J Clin Invest* 1994;93:2291-7.
9. Oz MC, Pinsky DJ, Koga S, Liao H, Marboe CC, Han D, et al. Novel preservation solution permits 24-hour preservation in rat and baboon cardiac transplant models. *Circulation* 1993;88[part 2]:291-7.
10. Naka Y, Chowdhury NC, Oz MC, Smith CR, Yano OJ, Michler RE, et al. Nitroglycerin maintains graft vascular homeostasis and enhances preservation in an orthotopic rat lung transplant model. *J Thorac Cardiovasc Surg* 1995;109:206-11.
11. Tanoue Y, Morita S, Ochiai Y, Hisahara M, Masuda M, Kawachi Y, et al. Inhibition of lipid peroxidation with the Lazaroid U74500A attenuates ischemia-reperfusion injury in a canine orthotopic heart transplantation model. *J Thorac Cardiovasc Surg* 1996;112:1017-26.
12. Tanoue Y, Morita S, Hisahara M, Tominaga R, Kawachi Y, Yasui H. Arresting donor hearts with extracellular-type cardioplegia prevents vasoconstriction induced by UW solution. *Cardiovasc Surg* 1998;6:622-8.
13. Ko W, Zelano J, Isom OW, Krieger KH. The effects of warm versus cold blood cardioplegia on endothelial function, myocardial function, and energetics. *Circulation* 1993;88[part 2]:359-65.
14. Baan J, van der Velde ET, de Bruin HG, Smeenk GJ, Koops J, van Dijk AD, et al. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. *Circulation* 1984;70:812-23.
15. Suga H. Ventricular energetics. *Physiol Rev* 1990;70:247-77.
16. Glower DD, Spratt JA, Snow ND, Kabas JS, Davis JW, Olsen CO, et al. Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. *Circulation* 1985;71:994-1009.
17. Little WC. The left ventricular dP/dt_{max} –end-diastolic volume relation in closed-chest dogs. *Circ Res* 1985;56:808-15.
18. Gaasch WH, Battle WE, Oboler AA, Banas JS Jr, Levine HJ. Left ventricular stress and compliance in man: with special reference to normalized ventricular function curves. *Circulation* 1972;45:746-62.
19. Ohishi N, Ohkawa H, Miike A, Tatano T, Yagi K. A new assay method for lipid peroxides using a methylene blue derivative. *Biochem Int* 1985;10:205-11.
20. Zeballos GA, Bernstein RD, Thompson CI, Forfia PR, Seyedi N, Shen W, et al. Pharmacodynamics of plasma nitrate/nitrite as an indication of nitric oxide formation in conscious dog. *Circulation* 1995;91:2982-8.
21. Glantz SA, Slinker BK. Two-factor analysis of variance with repeated measures on one factor. *Primer of applied regression and analysis of variance*. 1st ed. New York: McGraw-Hill; 1990. p. 404-31.
22. Weyrich AS, Ma X-I, Lefer AM. The role of L-arginine in ame-

- liorating reperfusion injury after myocardial ischemia in the cat. *Circulation* 1992;86:279-88.
23. Lefer AM. Attenuation of myocardial ischemia-reperfusion injury with nitric oxide replacement therapy. *Ann Thorac Surg* 1995;60:847-51.
24. Matheis G, Sherman MP, Buckberg GD, Haybron DM, Young HH, Ignarro LJ. Role of L-arginine-nitric oxide pathway in myocardial reoxygenation injury. *Am J Physiol* 1992;262:H616-20.
25. Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 1992;257:387-9.
26. Brady AJB, Poole-Wilson PA, Harding SE, Warren JB. Nitric oxide production within cardiac myocytes reduces their contractility in endotoxemia. *Am J Physiol* 1992;263:H1963-6.
27. Southorn PA, Powis G. Free radicals in medicine. I. Chemical nature and biologic reactions. *Mayo Clin Proc* 1988;63:381-9.
28. Zimmerman BJ, Granger DN. Reperfusion injury. *Surg Clin North Am* 1992;72:65-83.
29. Kojda G, Kottenberg K, Nix P, Schlüter KD, Piper HM, Noack E. Low increase in cGMP induced by organic nitrates and nitrovasodilators improves contractile response of rat ventricular myocytes. *Circ Res* 1996;78:91-101.
30. Feelisch M. The biochemical pathways of nitric oxide formation from nitrovasodilators: appropriate choice of exogenous NO donors and aspects of preparation and handling of aqueous NO solutions. *J Cardiovasc Pharmacol* 1991;17(Suppl 3):S25-33.

Targeted

The Journal of Thoracic and Cardiovascular Surgery gives you two tables of contents.

The condensed table of contents tells you at a glance what topics and authors are presented each month. The expanded table of contents gives you a brief abstract of each article. You select only those articles of most interest to you for further reading.