NITRIC OXIDE MEDIATES FLUID ACCUMULATION DURING CARDIOPULMONARY BYPASS

Kurt E. Wehberg, MD^a
Andrew H. Foster, MD^b
Robert M. Wise, MS^b
Joseph S. McLaughlin, MD^b
Martha J. Brunner, PhD^a

Fluid accumulation during cardiopulmonary bypass may be related to the production of endogenous vasoactive substances. We investigated the role of nitric oxide in mediating fluid accumulation during cardiopulmonary bypass. Normothermic cardiopulmonary bypass was carried out for 3 hours in male Sprague-Dawley rats with constant, nonpulsatile flow and hemodilution. Fluid accumulation (rate of change of external reservoir volume) was measured under three experimental conditions: saline solution control ($n = 8$), L-arginine infusion ($n = 6$), and *N*-nitro-L-arginine methyl ester infusion ($n = 6$). At the end of the experiments, body weight and organ wet/dry ratios were examined. Percentage weight gain was 77% greater in the *N*-nitro-L-arginine methyl ester group and 23% less in the L-arginine group compared with control values. Fluid accumulation was increased with *N*-nitro-L-arginine methyl ester after 30 minutes ($p < 0.01$) and reduced with L-arginine after 120 minutes ($p < 0.01$) compared with control animals. Water content was significantly decreased in the heart, lung, skin, muscle and peritoneum in rats receiving L-arginine. These data suggest that endogenous nitric oxide plays an important role in minimizing fluid accumulation during cardiopulmonary bypass. (*J Thorac Cardiovasc Surg* 1996;112:168-74)

Fluid accumulation is a significant problem in all patients undergoing cardiopulmonary bypass (CPB). This phenomenon has been characterized as a "whole-body inflammatory response" and may be related to the production and release of vasoactive substances that alter capillary permeability.¹⁻⁵ Nitric oxide (NO), which is produced in and released from the vascular endothelium, may play a key role in mediating this response.^{1, 6, 7}

NO is synthesized from L-arginine by the enzyme NO synthase, which may be competitively inhibited by L-arginine analogs such as *N*-nitro-L-arginine methyl ester (L-NAME).⁸ The physiologic functions of NO include the maintenance of vasodilatory tone,⁹ the mediation of cytokine release,⁸ and the modulation of microvascular permeability.¹⁰ The

function of NO during CPB, however, remains unknown.

The purpose of this study was to determine whether NO mediates fluid accumulation during CPB. In a rat model, we measured the rate of fluid accumulation during a 3-hour interval of CPB. The production of NO was either stimulated by L-arginine or inhibited by L-NAME.

Methods

Surgical preparation. Acute experiments were performed on healthy male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, Mass.) weighing 420 ± 20 gm. Experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). The protocol was approved by the Institutional Animal Care and Use Committee of the University of Maryland at Baltimore.

Rats were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg) and mechanically ventilated (Harvard Rodent Ventilator, model 683; Harvard Apparatus, Inc., South Natick, Mass.) through a tracheostomy. Additional sodium pentobarbital was given intravenously as needed (5 mg/kg) to maintain anesthesia throughout the experiment. The femoral artery and vein were cannulated to measure mean distal aortic pressure (MAP) and femoral venous pressure (FVP), respectively. Catheters were made of polyvinyl and Tygon tubing (Dural Plastics;

From the Departments of Surgery and Physiology^a and the Division of Thoracic and Cardiovascular Surgery,^b University of Maryland Medical System, Baltimore, Md.

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Address for reprints: Andrew H. Foster, MD, University of Maryland School of Medicine, Division of Cardiothoracic Surgery, Room N4W93, 22 S. Green St., Baltimore, MD 21201.

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Auburn, Australia) with an inner diameter of 0.5 mm and an outer diameter of 0.8 mm. After femoral catheterization, tracheostomy, and median sternotomy, the animals were heparinized (1000 units) and equilibration was allowed for 15 minutes before the treatment protocol was begun.

CPB was instituted under conditions of constant nonpulsatile flow, constant hemodilution, and normothermia. The pump-oxygenator unit (Fig. 1) consisted of a Masterflex (partially occlusive) roller pump (Cole-Parmer Instrument Co., Chicago, Ill.) and a noncompliant hollow-fiber membrane oxygenator (Travenol Laboratories, Inc., Deerfield, Ill.). Oxygenation was delivered at 250 ml/min with an inspired oxygen fraction of 50% to 75%. The total volume of the pump-oxygenator unit and tubing was 46 ml. The system was primed with 30 ml heparinized, homologous whole blood obtained from donor rats on the same day as the experiment. Venous return was drained by gravity into an open 60 ml reservoir from a miniaturized two-stage cannula placed through the right atrial appendage into the inferior vena cava (Varflex Syntholvar Tubing, 1.8 mm inner diameter; Rome, N.Y.). This system allowed optimal decompression of the right side of the heart. Oxygenated blood was filtered (40 μ m Pall Corp., Biomedical Products Div., Glen Cove, N.Y.) and then returned to the animal through an aortic cannula (Varflex Syntholvar, 1.4 mm inner diameter) that was placed through the left ventricular apex into the ascending aorta just distal to the aortic valve. The aortic cannula was anchored by a purse-string suture placed around and the apex of the heart. This inflow system bypassed the left ventricle and allowed any pulmonary venous and left atrial blood (although minimal) to be suctioned from the chest and returned to the venous reservoir. This technique also allowed coronary artery perfusion, as evidenced by the heart's continued beating throughout the experiment.

Fluid accumulation was measured as the rate of change of volume in the extracorporeal venous reservoir. This method, which has been described previously¹¹ but miniaturized, is an accurate estimation of fluid accumulation in the animal. The principle of this method is that any losses of volume in the external venous reservoir reflect reciprocal increases in volume in the animal. This principle holds true because the pump-oxygenator unit and tubing (closed system) are noncompliant. Evaporative losses and potential losses from the heat exchanger were deliberately controlled. Volume changes in the reservoir were measured by a pressure transducer (model 807; Beckman Instruments, Spinco Div., Palo Alto, Calif.) at the base of the external reservoir. Pressure was directly proportional to the height of the blood in the reservoir, and changes as small as 1 ml/min were accurately detected. Throughout the course of CPB, reservoir volume continually declined, reflecting fluid accumulation in the rat. Fluid (either homologous blood or lactated Ringer's solution) was replaced as needed to prevent the reservoir from emptying.

Factors known to affect fluid accumulation were held constant. Hemodilution, one of the most important factors in fluid accumulation,¹² was held constant by adding homologous blood to the external reservoir if hematocrit levels fell by 2%. Hematocrit levels were analyzed at

Fig. 1. CPB pump-oxygenator unit provides constant nonpulsatile flow and oxygenation through hollow-fiber membrane. Total volume of extracorporeal circuit is 46 ml. SVC, Superior vena cava; IVC, inferior vena cava.

30-minute intervals and were maintained at $13 \pm 1.2\%$. Pump flow was maintained at a constant level of $125 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Flow and hemodilution were predetermined to provide adequate tissue oxygenation in the rat during CPB on the basis of several metabolic parameters, including arterial pH (maintained at 7.37 ± 0.02), venous oxygen saturation (range 70% to 86%), and arterial oxygen tension (maintained at $209 \pm 12 \text{ mm Hg}$). Osmolality remained constant throughout the experiments ($274 \pm 2 \text{ mmol/L}$ before operation to $279 \pm 9 \text{ mmol/L}$ at 180 minutes, with no significant change). Resistance in the tubing of the venous outflow tract, an important determinant of venous hydrostatic pressure, could also affect fluid accumulation. The height of the venous outflow cannula was therefore held constant at 10 cm below the level of the right atrium to maintain right atrial pressure at a constant level. Blood drained by gravity into the venous reservoir below. Body temperature was maintained at between 36° to 38° C by a heating blanket under the animal and a heating lamp above the animal.

Protocol. Rats were randomly assigned to three treatment groups. Control animals ($n = 8$) received a 3 ml saline solution bolus (vehicle control) 15 minutes before initiation of CPB. Animals in the L-arginine group ($n = 6$) received L-arginine hydrochloride (Sigma Chemical Company, St. Louis, Mo.) as an intravenous bolus of 150 mg/kg 15 minutes before CPB and an infusion of $30 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ during CPB. Animals in the L-NAME group ($n = 6$) received L-NAME (Sigma) as a bolus of 30

Fig. 2. Fluid accumulation during CPB. Significant differences between control and L-NAME groups ($p < 0.01$) at specific time points represented by *asterisks*; significant differences between control and L-arginine ($p < 0.01$) represented by *plus signs*.

mg/kg before CPB and an infusion of $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{hr}^{-1}$ during CPB.

CPB was maintained for 3 hours. Fluid uptake, femoral venous pressure, and MAP were measured at 0, 15, 30, 60, 90, 120, and 180 minutes. Data points represent the average level during the last 2 minutes of each time interval. The time designated as -30 minutes represents baseline data before the experimental drug was given, -15 minutes represents the time at which the bolus of the experimental drug was given and the infusion was started, and 0 minutes represents the time at which the oxygenated blood was initiated through the aortic cannula. To evaluate fluid uptake, MAP, and FVP within each experimental group as functions of CPB time, comparisons were made between the original data point (e.g., for fluid uptake measurement the first data point for L-NAME was at 15 minutes, whereas for FVP and MAP the first data points were at -30 minutes) and successive data points were within the experimental group. After the final measurement at 180 minutes, the animal was killed and its postoperative body weight was recorded. Blood and intraluminal contents were removed from organs before post mortem wet weights were obtained. The organs were then dried at 60°C for 24 hours and reweighed (dry weight) for the calculation of wet/dry ratios (wet weight minus dry weight divided by dry weight, expressed as milliliters of fluid per gram of tissue). Organ weights of animals undergoing CPB were compared with those of animals who underwent anesthesia, median sternotomy, heparinization, and constant hemodilution without CPB for 3 hours ("sham-operated group," $n = 6$).

Statistical analyses. All data are expressed as mean $\pm$ standard error of the mean. Analysis of variance with

repeated measures was used to evaluate differences in fluid accumulation, weight gain, and arterial and venous pressures as effects of time (response) and treatment group (control, L-arginine, and L-NAME groups). The Newman-Keuls test for individual differences was used to determine differences within groups. Organ weights were compared between treatment groups by means of a one-way analysis of variance. Significant differences were noted at $p < 0.05$.

Results

Fig. 2 illustrates the rate of fluid accumulation ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) during 3 hours of CPB. The rate of fluid accumulation was significantly greater in rats with NO inhibition (L-NAME) than in control rats after 30 minutes ($p < 0.01$). The rate of fluid accumulation in the L-arginine group was significantly less than in the control group after 120 minutes ($p < 0.01$). The average rate of fluid accumulation through 3 hours was significantly greater ($p < 0.01$) in the L-NAME group ($8.1 \pm 2.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) than in the control group ($5.0 \pm 0.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and was significantly less ($p < 0.001$) in the L-arginine group ($3.0 \pm 0.5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Fluid accumulation as a function of time was significantly increased after 120 minutes in the control group ($p < 0.05$) and significantly increased after 30 minutes in the L-NAME group ($p < 0.01$). Fluid accumulation as a response to time was not

Fig. 3. Percentage weight gain after 3 hours of CPB. Note that weight gain was significantly less with L-arginine ($p < 0.05$ vs control, represented by plus sign) and greater with L-NAME ($p < 0.01$ vs control represented by asterisk).

Table I. Hemodynamic data during CPB: role of nitric oxide

Time (min)	MAP (mm Hg)			FVP (mm Hg)		
	Control	L-Arginine	L-NAME	Control	L-Arginine	L-NAME
-30	85.6 ± 8.2	78.3 ± 5.9	82.0 ± 5.9	2.2 ± 0.6	1.1 ± 0.3	1.8 ± 0.9
-15	85.0 ± 8.0	71.7 ± 9.4	112.0 ± 4.9*	2.6 ± 0.7	1.0 ± 0.3†	1.7 ± 0.9
0	72.0 ± 12.1	90.0 ± 14.9	138.3 ± 22.9*	4.2 ± 1.1	1.2 ± 0.2†	3.0 ± 1.1
15	60.0 ± 12.0	105.0 ± 17.4	139.0 ± 21.6*	4.4 ± 1.1	0.9 ± 0.3†	3.9 ± 1.2
30	64.4 ± 12.4	78.3 ± 17.9	107.0 ± 25.1*	3.9 ± 0.8	1.9 ± 0.3†	4.9 ± 1.2
60	73.3 ± 13.4	65.0 ± 20.7	93.0 ± 16.4	4.7 ± 0.6	1.6 ± 0.6†	9.3 ± 1.6*
90	93.6 ± 15.6	64.2 ± 27.9†	93.0 ± 10.6	5.2 ± 1.1	2.3 ± 0.4†	10.4 ± 1.3*
120	112.6 ± 16.6	64.7 ± 24.4†	98.3 ± 10.2	7.7 ± 1.1	2.7 ± 0.7†	10.0 ± 2.7*
180	117.9 ± 5.4	67.5 ± 25.8†	90.0 ± 15.8	7.4 ± 1.4	2.9 ± 1.2†	12.0 ± 2.0*

*Significant difference between control and L-NAME groups.

†Significant difference between control and L-arginine groups.

significant in the L-arginine group. Percentage weight gain (Fig. 3) was also significantly greater with L-NAME ($p < 0.01$) and significantly less with L-arginine ($p < 0.01$) than control values.

Hemodynamic parameters during CPB are shown in Table I. In the control group, MAP decreased rapidly after the initiation of bypass ($p < 0.01$, -15 minutes vs 15 minutes), returned to baseline levels, and was not significantly increased with respect to time after 30 minutes. In the L-NAME group, MAP was significantly elevated compared with control values during the first 30 minutes of CPB ($p < 0.01$). MAP was not different from preoperative levels after 30 minutes, however, nor was it significantly different from control values after 30 minutes. In the

L-arginine group, MAP was significantly decreased compared with control values after 90 minutes ($p < 0.01$). The response of MAP to time in the L-arginine group was not significant. FVP was significantly increased in the L-NAME group after 60 minutes ($p < 0.001$) and decreased in the L-arginine group compared with the control group ($p < 0.01$) at all time points except for the pre-CPB time points (-15 minutes and -30 minutes). The response to FVP as a function of time was significant in the L-NAME group after 60 minutes ($p < 0.001$) but was not significant in the L-arginine or control groups.

Organ wet-dry ratios normalized for tissue weight after 3 hours CPB are listed in Table II. Fluid accumulation was increased compared with that in

Table II. Organ wet/dry ratios (normalized for weight) after 3 hours of CPB

Organ	Sham (no CPB)	Control (CPB)	L-NAME (CPB)	L-Arginine (CPB)
Heart	3.49 ± 0.16	3.95 ± 0.15*	4.16 ± 0.14	3.58 ± 0.09†
Lung	4.14 ± 0.36	6.46 ± 0.20*	6.04 ± 0.20	5.54 ± 0.30†
Brain	2.95 ± 0.28	4.01 ± 0.13*	3.64 ± 0.21	3.84 ± 0.20
Kidney	2.98 ± 0.17	4.43 ± 0.08*	4.53 ± 0.19	4.53 ± 0.11
Liver	2.22 ± 0.01	2.49 ± 0.21	2.73 ± 0.29	2.26 ± 0.13
Spleen	3.16 ± 0.13	3.85 ± 0.29*	3.62 ± 0.11	4.60 ± 0.82
S. Bowel	2.85 ± 0.12	3.97 ± 0.37*	3.93 ± 0.12	3.91 ± 0.26
Colon	2.62 ± 0.20	8.76 ± 0.71*	8.17 ± 0.44	8.70 ± 0.64
Muscle	2.79 ± 0.14	3.17 ± 0.19	3.96 ± 0.21‡	3.49 ± 0.20
Skin	1.47 ± 0.05	1.47 ± 0.35	2.70 ± 0.24‡	2.41 ± 0.15

Data expressed in milliliters of fluid per gram of tissue.

*Significant difference between control and sham operated groups.

†Significant difference between control and L-arginine groups.

‡Significant difference between control and L-NAME groups.

the sham-operated group in all organs of control animals except skin, muscle, and liver. Although CPB itself had no effect on fluid accumulation in skin and muscle, inhibition of NO by L-NAME markedly increased water content in these organs. Organ fluid accumulation was significantly decreased in the L-arginine group compared with the control group in the heart, lung, skin, and muscle. To estimate the approximate sensible (ascites) and insensible fluid losses, we compared the total amount of fluid given through 3 hours to the total weight gained. If more fluid was given than weight gained, this amount should represent insensible plus sensible losses. The control group had 71 ± 13 ml fluid loss, the L-NAME group had 230 ± 26 ml, and the L-arginine group had 15 ± 7 ml sensible and insensible losses. These data were consistent with our clinical observation that animals receiving L-NAME had massive ascites and those receiving L-arginine had virtually no ascites.

Discussion

CPB may be associated with massive fluid accumulation.¹ Our data show that systemic inhibition of the endogenous NO system by L-NAME leads to increased fluid accumulation, whereas enhancement of NO synthesis by L-arginine leads to reduced fluid accumulation during CPB. Accordingly, percentage weight gain increases with L-NAME and decreases with L-arginine. These results suggest that endogenous NO plays an important role in mediating fluid accumulation during CPB.

The release of vasoactive substances during CPB has been well documented. Endothelin-1,^{3,4} prostaglandin E₂ and prostacyclin,¹³ interleukin-1,² bradykinin,⁵ and complement C3a and C5a¹⁴ levels have

been shown to be increased during CPB. These substances interact with the endothelium to cause marked inflammatory responses, which lead to edema formation and fluid accumulation.¹ NO, which is produced and released by the endothelium, has not yet been previously described as a modulator of fluid accumulation during CPB.

Fluid accumulation is directly proportional to the duration of CPB.¹¹ In the control and L-NAME groups in this study, fluid accumulation increased progressively with time. The control animals had significant fluid accumulation as a function of time after 120 minutes, whereas animals receiving L-NAME had increased fluid accumulation after 30 minutes. More important, animals in the L-arginine group did not have increased fluid accumulation after 120 minutes, suggesting that NO stimulation minimized the progressive fluid accumulation. These findings suggest that NO may be an important factor in preventing excessive fluid accumulation at longer CPB durations.

In our study, L-NAME caused marked elevations in FVP. It is well accepted that venous capacitance is decreased during and early after CPB.¹⁵ Suppressed NO formation could be a mechanism through which decreased venous capacitance occurs during CPB. This phenomenon is supported by Glick and associates,¹⁶ who demonstrated a dose-dependent increase in mean systemic filling pressure and reduction in venous capacitance with increasing doses of N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of NO like L-NAME. Furthermore, our additional findings of significant reductions in FVP with L-arginine, which promotes NO formation, confirms that NO decreases venous tone during CPB. These results and those of previous studies

suggest that NO may play a crucial role in decreasing venous tone during CPB.

The mechanism by which NO mediates fluid accumulation during CPB may be related to its action on hydrostatic pressure in the arterial and central venous system and possibly on lymphatic drainage. MAP was significantly elevated with L-NAME only early in CPB. Because MAP was not significantly increased with L-NAME (compared with control values) in late CPB, the hydrostatic effects of MAP may not be the primary determinants of fluid accumulation in late CPB. On the contrary, increased fluid accumulation in the L-NAME group in late CPB may be a direct result of the significantly elevated venous hydrostatic pressure. Finally, the increased fluid accumulation in the L-NAME group may be related to NO's ability to modulate lymphatic drainage, which has been suggested by recent studies.¹⁷ Our concomitant findings of increased fluid accumulation and marked elevations in FVP at longer CPB durations suggest that NO is important in minimizing excessive fluid loss by its vasodilatory actions on the venous bed.

Another mechanism by which fluid accumulation is increased during CPB could be through NO's effect on microvascular permeability. Kubes and Granger¹⁰ recently demonstrated that inhibition of NO production by L-NAME leads to an increase in microvascular permeability in the small intestine of the cat. They also showed that L-NAME increases precapillary and postcapillary resistance, although it does not change net capillary pressure, suggesting that NO affects permeability alone without changes in capillary pressure. Furthermore, they found that L-arginine prevented the increase in microvascular permeability and concluded that NO plays an important role in minimizing loss of fluid from the vasculature. Our results support this conclusion and suggest that NO may be critically important in maintaining capillary integrity during CPB.

Recent evidence suggests that NO may contribute to the inflammatory response and edema formation in specific organs. In the small bowel of the cat, inhibition of NO by L-NAME causes a rapid leakage of fluid out of the vasculature and into the interstitial space.¹⁰ We have shown that organ fluid accumulation is increased as a response to CPB alone (control group) in almost all organs except skin, muscle, and liver. Organ water content in the skin and muscle was markedly increased in the L-NAME group. Another interesting observation we found was that animals receiving L-NAME had massive

ascites during CPB, whereas the animals receiving L-arginine had virtually no ascites. This suggests that NO metabolism in the endothelium of mesenteric vasculature is important in minimizing ascites and sensible fluid loss during CPB.

Fluid accumulation in human patients during CPB is variable and has been estimated at between 1 and 2.5 L during the 1- to 3-hour CPB time,¹² which yields an average rate of accumulation of approximately $0.5 \text{ to } 1.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Similar rates have been found in normotensive dogs undergoing CPB, although the rate was increased fivefold in hypertensive dogs.¹¹ The quantitative rate of fluid uptake ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in the control rats in our study is higher than in previous studies. Differences in levels in our study may be explained by the moderate to extreme hemodilution to 13% (as compared with 15% to 25% in human beings and no hemodilution in the dog study) and the higher resistance in the venous outflow tubing because of its smaller diameter to accommodate the rat atrium (1 to 2 mm). Additionally, age differences (3-month-old rats vs 2- to 3-year-old dogs vs 60-year-old human patients) may account for quantitative differences in rates of fluid accumulation. In any case, differences in fluid accumulation within our study were attributed to changes in treatment protocol.

In summary, we have demonstrated that the endogenous NO system is an important mediator of fluid accumulation during CPB. This vasoactive substance appears to minimize whole-body fluid accumulation during CPB and may play an important role in preventing ascites as well as in reducing fluid content in the heart, lung, skin, and muscle during CPB. This study suggests that therapeutic modalities that enhance endogenous NO production may be beneficial in reducing whole-body and specific organ fluid accumulation during CPB. Specific mechanisms through which NO mediates fluid accumulation during CPB remain to be elucidated.

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