DEOXYGENATED BLOOD MINIMIZES ADHERENCE OF SONICATED ALBUMIN MICROBUBBLES DURING CARDIOPLEGIC ARREST AND AFTER BLOOD REPERFUSION: EXPERIMENTAL AND CLINICAL OBSERVATIONS WITH MYOCARDIAL CONTRAST ECHOCARDIOGRAPHY

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Both administration of cardioplegic solution and blood reperfusion result in endothelial dysfunction. The transit rate of albumin microbubbles during myocardial contrast echocardiography may reflect endothelial injury. Accordingly, we performed myocardial contrast echocardiography in 12 dogs undergoing cardiopulmonary bypass and measured the myocardial transit rate of microbubbles injected into the aortic root during delivery of cardioplegic solutions containing arterial and venous blood and delivery of pure crystalloid cardioplegic solution. The myocardial transit rate of ^{99m}Tc-labeled red blood cells was measured and perfusates were sampled for biochemical analysis at each stage. The microbubble transit rate was markedly prolonged during delivery of crystalloid cardioplegic solution and improved significantly during infusion of blood cardioplegic solution ($p < 0.001$); venous compared with arterial blood in the solution resulted in a greater rate ($p < 0.001$). The microbubble transit rate did not correlate with pH, oxygen tension or carbon dioxide tension values, or K^+ concentration. The red blood cell transit rate remained constant regardless of the cardioplegic perfusate infused. Myocardial contrast echocardiography was also performed in 12 patients undergoing coronary artery bypass who underwent sequential arterial and venous reperfusion after cardioplegic arrest. The microbubble transit rate was faster with venous than arterial blood reperfusion ($p = 0.01$), although this gain was diminished when arterial blood reperfusion preceded venous blood reperfusion ($p = 0.05$). Our results indicate that endothelial dysfunction after cardioplegic arrest may be ameliorated by reperfusion with venous rather than arterial blood. (J Thorac Cardiovasc Surg 1997;113:1100-8)

Although crystalloid cardioplegic solutions protect the myocardium during cardiopulmonary bypass (CPB), they can independently cause coronary microvascular endothelial injury.[1-4] Abnormalities in endothelial function may occur even when the endothelium does not demonstrate any ultrastructural changes.[5] More profound functional and histologic injury may be seen after reperfusion,[4, 6, 7] which is largely attributable to cellular inflammatory responses[8] and accumulation of oxygen-derived free radicals.[7, 9-11] Addition of blood to cardioplegic solutions has been shown to reduce endothelial damage.[1, 5, 12, 13] During reperfusion, oxygen-derived free radicals are generated, and thus controlled reperfusion with gradual reoxygenation after CPB has been proposed to decrease endothelial injury in previously hypoxic hearts.[14, 15] Because of the difficulty in assessing endothelial function, however,

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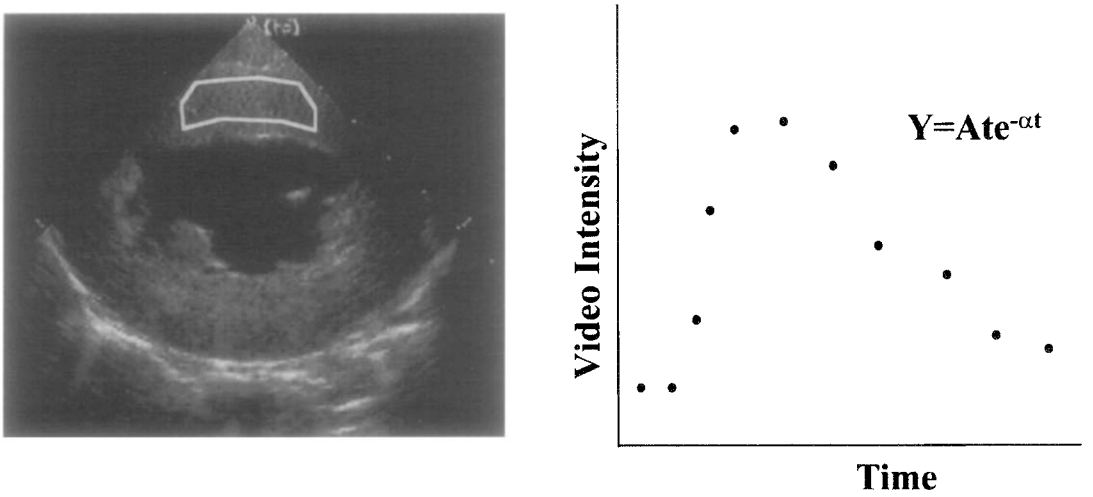

Fig. 1. Illustration of method for obtaining time-intensity data during MCE. A contrast-enhanced left ventricular short axis image is shown with a region of interest placed over the anterior myocardium. The time-intensity plot from that region is shown at the *right,* and a γ-variate function is fitted to the data after background subtraction to derive mean transit rate. See text for explanation of equation variables.

studies designed to determine the effects of oxygen tension (Po_2) during perfusion of blood cardioplegic solution and reperfusion have been confined either to the evaluation of indirect indices of vascular function or to ex vivo microvascular preparations.

Myocardial contrast echocardiography (MCE) has been used intraoperatively to assess myocardial perfusion.[16-18] In intraoperative MCE, sonicated albumin microbubbles, which cause backscatter of ultrasound, are injected into the aortic root and their transit through the myocardium is recorded during simultaneously performed echocardiography. In a blood-perfused, beating heart, the rheologic features of sonicated albumin microbubbles are similar to those of red blood cells (RBCs).[19, 20] During delivery of crystalloid cardioplegic solution, however, the transit of these microbubbles is markedly prolonged. Microvascular studies have suggested this effect may be caused by microbubble adherence to the venular endothelium, possibly reflecting endothelial injury.[21]

We hypothesized that, because of its low Po_2, venous blood would result in less microvascular injury on reperfusion compared with arterial blood and therefore its use would result in less prolongation in the microbubble transit rate seen during MCE. We tested this hypothesis both in dogs and in patients undergoing coronary artery bypass.

Methods

Animal experimental preparation. The study protocol was approved by the animal research committee at the University of Virginia and conformed to the "Principles of Laboratory Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources. Twenty-four male mongrel dogs were used in the study. Twelve of these dogs served as blood donors and the remaining 12 (mean weight 27 ± 1 kg) served as the study animals. These study dogs were anesthetized with 30 $mg \cdot kg^{-1}$ sodium pentobarbital and intubated and the lungs mechanically ventilated with use of a respirator pump (model 607, Harvard Apparatus, South Natick, Mass.). Additional anesthesia was administered during the experiment as needed. Catheters were placed in the right femoral artery for pressure measurement and in the right femoral vein for administration of fluids and drugs as needed. A median sternotomy was performed and the heart was suspended in a pericardial cradle. Heparin (300 $U \cdot kg^{-1}$) was administered intravenously and supplemented every 90 minutes.

The superior vena cava and right ventricle were cannulated transatrially. The left femoral artery was cannulated and CPB was instituted with use of a roller pump (model 6002, Sarns Inc., Ann Arbor, Mich.) and a membrane oxygenator (American Bentley Corp., Irvine, Calif.). Blood flow through the pump was maintained at 2.0 to 3.0 $L \cdot min^{-1}$ and the systemic blood temperature was maintained at 37° C by means of a blood warmer (Blanketrol, Subzero Products Inc., Cincinnati, Ohio). An infusion catheter (DLP Inc.) was placed in the ascending aorta and its side arm was used to monitor aortic root pressure. The main port was connected via one arm of a Y connector to

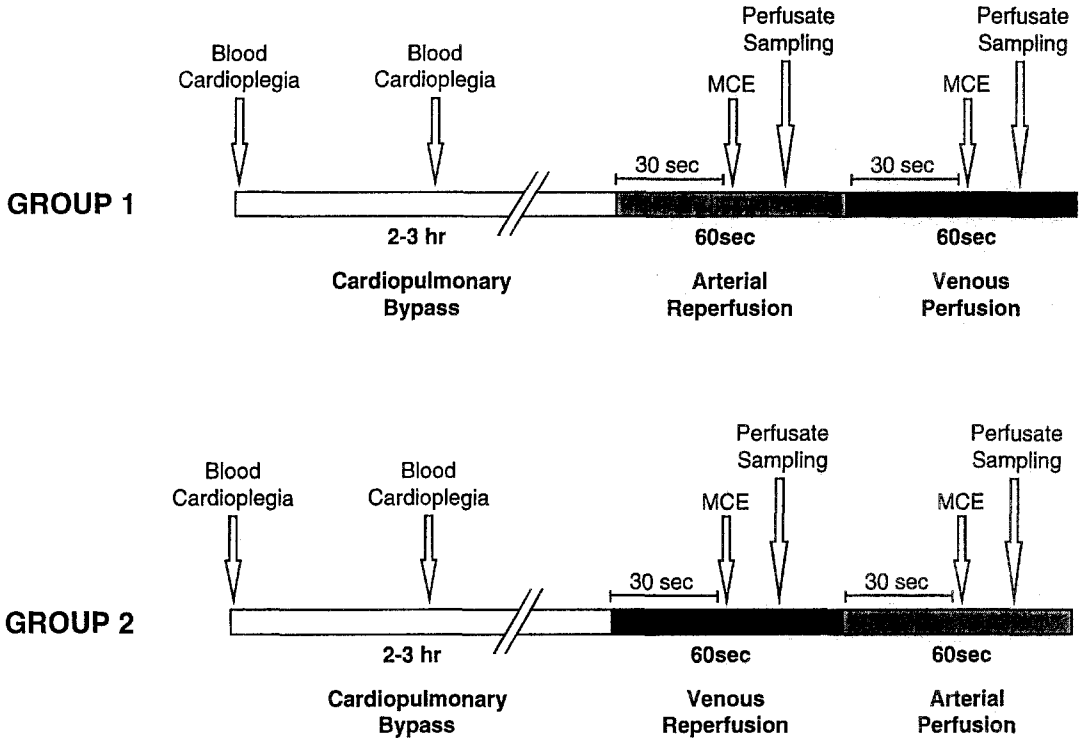

Fig. 2. Patient study protocol. Patients were randomized to receive either arterial (group 1) or venous (group 2) blood first during reperfusion.

a power injector (Angiomat 3000, Liebel-Flarsheim, Cincinnati, Ohio) for injection of microbubbles and radiolabeled RBCs. The other arm was used for delivery of perfusate at a constant rate of 150 ml · min^{-1} into the aortic root by means of a roller pump (model S 10K II, Sarns Inc.) connected to a mixing reservoir (model CPS4000, Gish Biomedical, Irvine, Calif.). This reservoir allowed for the constitution of different cardioplegic solution mixtures and contained a heating/cooling coil to maintain constant temperature. Arterial or venous blood from the bypass circuit was added to the reservoir via side limbs. A sampling port in the bottom of the chamber allowed collection of perfusate for analysis.

All pressure lines were connected to a multichannel recorder (model 4568C, Hewlett-Packard, Andover, Mass.) via fluid-filled transducers (model 1295A, Hewlett-Packard). The surface electrocardiogram and core body temperature were also monitored and recorded.

Patient study. Twelve consecutive patients (10 men, 2 women) referred for coronary artery bypass were included in the study. The study protocol was approved by the investigational review board at the University of Virginia and all patients gave written informed consent. Exclusion criteria were hypersensitivity to any blood product, prior coronary artery bypass, unstable operative hemodynamic condition, or more than mild aortic insufficiency on contrast echocardiography.

CPB was established after heparin administration (300 U · kg^{-1}) with single right atrial and ascending aortic cannulation. The extracorporeal circuit comprised a heart-lung pump system (System 8000, Sarns Inc. and Bio-Medicus 540, Minneapolis, Minn.) placed in series with a hollow-fiber oxygenator with a closed venous reservoir (Univox, Baxter/Bentley, Irvine, Calif.) and an arterial line filter. Nonpulsatile flow rates from 2.5 to 5.0 L · min^{-1} were used in the extracorporeal circuit to maintain mean systemic pressures greater than 60 mm Hg, and systemic temperatures were maintained between 25° and 37° C.

After the aorta was crossclamped, cardioplegia was achieved with use of a 1:1 dilution of oxygenated blood containing KCl (20 mEq · L^{-1}) and crystalloid cardioplegic solution (Plegisol, Abbott Laboratories, North Chicago, Ill., activated with 25 mEq · L^{-1} sodium bicarbonate) at a temperature of 6° to 8° C. The cardioplegic solution was administered in an anterograde fashion in all patients via an aortic root cannula (DLP) at an initial rate of 250 to 400 ml · min^{-1} to achieve cardiac arrest. Additional cardioplegic solution was administered after each graft anastomosis or if cardiac mechanical activity was visualized during the period of cardiac arrest.

MCE. MCE was performed with a phased-array system (Sonos 2500, Hewlett-Packard) equipped with a 5 MHz transducer. A saline bath placed between the heart and the transducer was used as an acoustic interface in the canine studies. In patients, the transducer was placed in a sterile sleeve and positioned directly on the anterior cardiac surface. Images were acquired in the short-axis view at the mid–papillary muscle level. Gain settings were adjusted at the beginning of each experiment and were

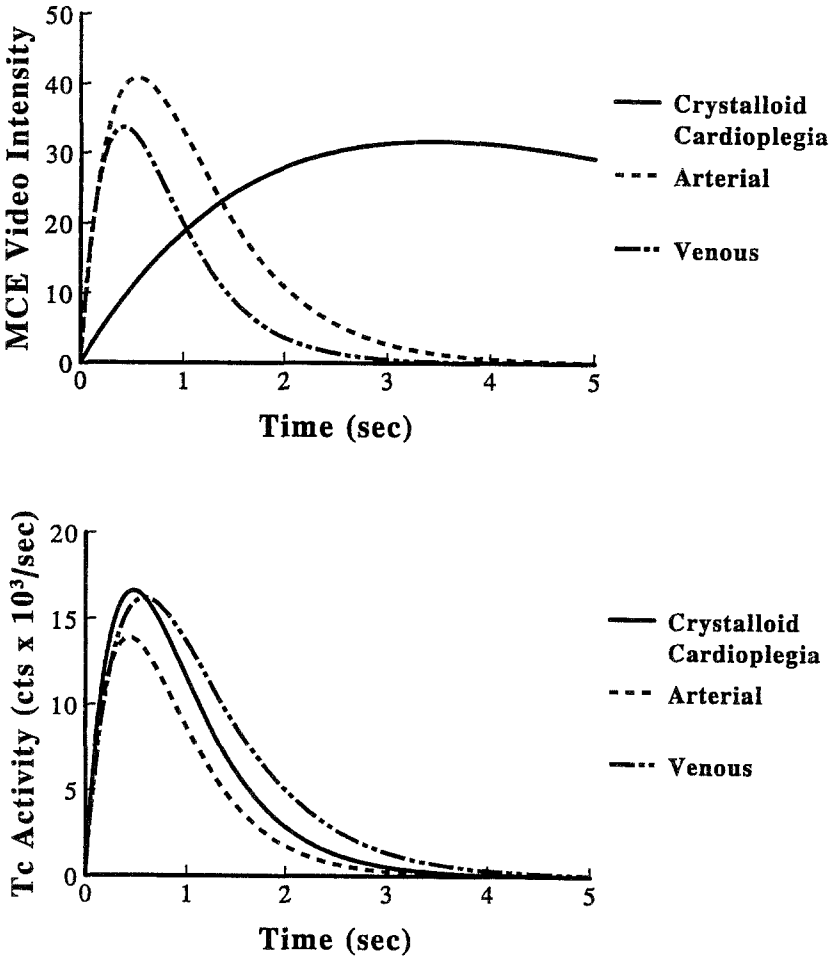

Fig. 3. Examples of MCE time-intensity curves *(top panel)* and ^{99m}Tc-labeled RBC time-activity curves *(bottom panel)* from one dog. See text for details. *cts,* Counts.

held constant throughout. The maximal dynamic range (60 dB) was used. Images were recorded on 1.25 cm videotape at 30 Hz with use of a high-fidelity recorder (Panasonic AG6200, Matsushita Electric, Japan).

Sonicated albumin microbubbles (Albunex, Molecular Biosystems Inc., San Diego, Calif.) with a mean size of 4.3 μm and a concentration of $0.5 \cdot 10^9$ ml^{-1} were used for MCE. In the animal studies, 1 to 1.5 ml of Albunex microbubbles diluted to a total volume of 2 ml with 0.9% NaCl was used. In the human studies, 1.5 to 3.0 ml of Albunex microbubbles diluted to a total volume of 10 ml with 0.9% NaCl was used. Microbubbles were power injected (Angiomat 3000, Liebel-Flarsheim, for animal studies and Mark 5-plus, MedRad, Pittsburgh, Pa., for human studies) into the aortic root via the DLP catheter at a rate of 5 ml $\cdot$ sec^{-1}.

Data were analyzed off-line with use of previously described algorithms.[22] Images encompassing the period from just before contrast injection until the disappearance of contrast from the myocardium were transferred to the video memory of a computer (Mipron, Kontron Electronics, Eching, Germany) in a 244 $\times$ 244 $\times$ 8 bit format. A region of interest was defined over a myocardial segment (but not over the anterior myocardium if a left internal thoracic graft was used) and the average videointensity in this region was measured in every fifth frame (Fig. 1). The time-intensity data were background subtracted and fitted to a γ-variate function: $y = Ate^{-\alpha t}$, where A is a scaling factor, t is time, and α is the mean transit rate.[22]

Radiolabeled RBC acquisition. ^{99m}Tc-labeled RBCs from the donor dog were used to measure the RBC transit rate. Approximately 0.1 mCi of labeled RBCs was diluted to a total volume of 2 ml and injected into the aortic root via the DLP catheter at a rate of 5 ml $\cdot$ sec^{-1}. Radioactivity within the myocardium was measured with use of a miniature cesium iodide probe (Oxford Instruments, Oxford, England) equipped with a converging collimator placed 3 cm above the anterior cardiac surface. The photodiode of the probe was connected to a preamplifier-amplifier unit designed specifically to detect the ^{99m}Tc photopeak. Data were acquired at a sampling rate of 2 Hz and transferred to a personal computer (model 310, Dell Computer Corp., Austin, Tex.). Time activity data were

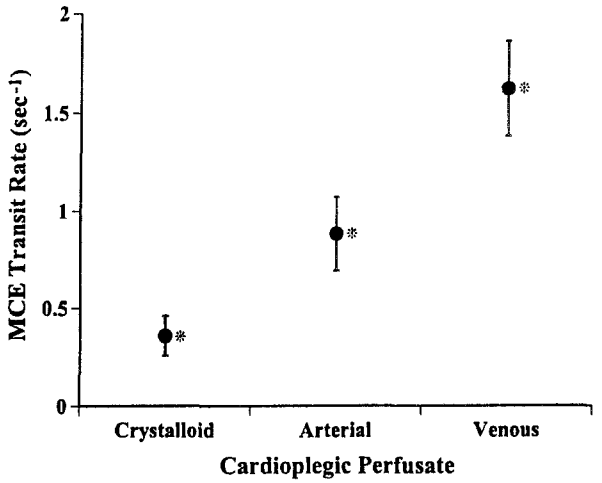

Fig. 4. Mean (plus or minus standard error of the mean) microbubble transit rates from all 12 dogs during perfusion with various cardioplegic solutions. $^*p < 0.05$ compared with all other data points.

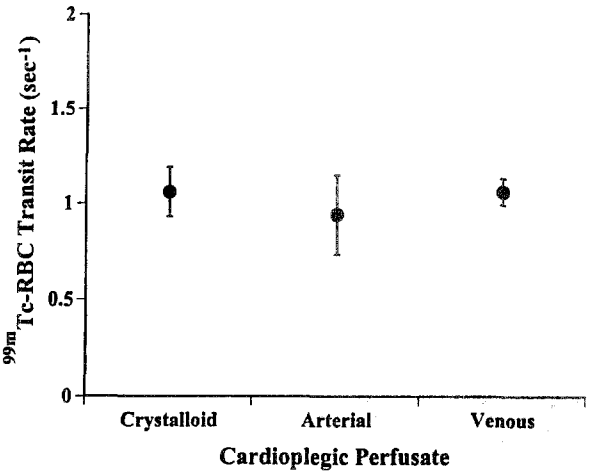

Fig. 5. ^{99m}Tc-labeled RBC transit rates from all 12 dogs during perfusion with various cardioplegic solutions.

fitted to a γ-variate function and, similar to the method of MCE analysis, α corresponded to the mean transit rate.

Animal protocol. After the aorta was crossclamped, arterial blood containing KCl (20 mEq · L^{-1}) was administered through the aortic root at a rate of 150 ml · min^{-1} for approximately 2 minutes to achieve cardiac arrest. MCE and labeled RBC data were acquired in random order approximately 1 minute after cardiac arrest was achieved, and blood was obtained from the mixing chamber for analysis of K^+ concentration, osmolality, pH, and P_{O_2} and carbon dioxide tension (P_{CO_2}) values. The crossclamp was removed and similar steps were repeated with venous blood and arterial blood and with crystalloid cardioplegic solution (Plegisol) in a random order. Crystalloid cardioplegic solution was activated before use by addition of 20 ml · L^{-1} of 8.4% $NaHCO_3$, and supplemental KCl was added to each perfusate as needed to maintain concentrations of approximately 20 mEq · L^{-1}.

Human experimental protocol. The protocol for the human studies is depicted in Fig. 2. After completion of the final graft anastomosis, the heart was reperfused at a constant flow rate for 60 seconds with normothermic arterial or venous blood, as randomly assigned. MCE was performed approximately 30 seconds after reperfusion and samples were taken from a side arm of the perfusion line to measure K^+ and total protein concentrations, pH, P_{O_2} and P_{CO_2} values, and hematocrit level. The perfusate was then switched to the alternate blood composition (arterial or venous) for 60 seconds and both MCE and sampling of the perfusate were repeated.

Statistical methods. Data were analyzed with RS/1 and SAS programs and are expressed as the mean plus or minus 1 standard deviation unless otherwise specified. In animals, comparisons between mean transit rates for different perfusates and comparisons between perfusate variables were made with repeated-measures analysis of variance. In patients, comparisons of MCE data between arterial and venous perfusion were made with the Wilcoxon signed-rank test. Interval comparisons were made with the unpaired Student's *t* test.

Results

Animal studies. Illustrated in the *top panel* of Fig. 3 are examples from one dog of background-subtracted time-intensity curves obtained with MCE during perfusion with various cardioplegic solutions. Microbubbles persisted in the myocardial microcirculation during delivery of crystalloid cardioplegic solution, which resulted in a wide curve and a slow mean transit rate. During infusion of blood cardioplegic solution, the transit rate increased, as reflected by a narrowing of the time-intensity curves, and was faster with venous compared with arterial blood cardioplegic solution. The transit rate of labeled RBCs was not prolonged during crystalloid cardioplegic solution delivery and remained constant during infusion of the blood-containing perfusates, as illustrated by the similar time-activity curves in the *bottom panel* of Fig. 3.

MCE data from all 12 dogs paralleled the findings in the example discussed in the previous paragraph and are shown in Fig. 4. During delivery of crystalloid cardioplegic solution, persistence of microbubbles in the myocardium resulted in a slow transit rate. The microbubble transit rate was significantly increased during delivery of arterial blood cardioplegic solution. Deoxygenated venous blood cardioplegic solution resulted in an even faster transit rate. Radiolabeled RBC transit rates (Fig. 5) were

similar with crystalloid, arterial blood, and venous blood cardioplegic solutions.

Hemodynamic and biochemical variables measured for each perfusate are presented in Table I. There were no statistically significant differences in the aortic root pressure, coronary vascular resistance, K^+ concentration, osmolality, pH, Pco_2 level, or perfusate temperature. Significant differences in the Po_2 values were found among all three perfusates. Normal sinus rhythm before aortic crossclamping and cardioplegic solution delivery occurred in 40%, 36%, and 27% of the animals for stages that used arterial blood, venous blood, and pure crystalloid cardioplegic solution, respectively ($p = 0.39$).

Patient studies. Demographic and clinical data are presented in Table II. The characteristics of patients who received arterial blood reperfusion first were similar to those of patients who received venous blood reperfusion first, with the exception of a longer aortic crossclamp time in the former.

MCE transit rate data for all 12 patients are illustrated in Fig. 6. Regardless of the sequence of reperfusion, venous blood resulted in faster transit rates compared with those for arterial blood in all patients. The mean microbubble transit rate was 0.97 ± 0.39 sec^{-1} during arterial blood perfusion and 1.78 ± 0.71 sec^{-1} during venous blood perfusion ($p = 0.01$). Venous blood resulted in a faster transit rate when given before arterial blood (group 2), although this finding was of borderline significance ($p = 0.05$). No relationship was found between the MCE transit rate and perfusate sequence (that is, between first and second blood perfusate). Comparison of the chemical analysis of the perfusates is shown in Table III. Both the pH and Po_2 values were lower in venous compared with arterial blood.

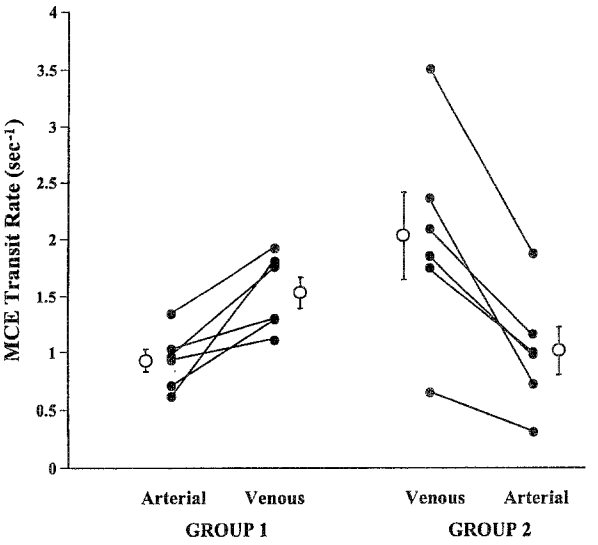

Fig. 6. Microbubble transit rates obtained from patients during arterial and venous blood reperfusion after cardioplegic arrest.

Discussion

In our canine model of CPB, we have demonstrated that sonicated albumin microbubbles persist in the myocardium during administration of crystalloid cardioplegic solution. RBC transit rates during delivery of crystalloid cardioplegic solution, however, remain unaltered compared with those during blood cardioplegic solution delivery, which suggests that the persistence of the MCE contrast effect reflects a specific and unique interaction between microbubbles and the microvascular endothelium. The use of blood-containing cardioplegic perfusates improved the transit rate and a greater effect was observed with venous than with arterial blood. Similar results were found in patients undergoing reperfusion after cardioplegic arrest and CPB.

Endothelial injury during cardioplegic arrest and reperfusion. Reperfusion of ischemic myocardium results in functional impairment of the vascular endothelium, which is most marked in vessels smaller than 200 μm.[23] These abnormalities may occur even in the absence of histologic evidence of injury,[9, 23] although findings of endothelial cell edema or disruption are not uncommon and may be related to the length of time after reperfusion.[10, 24] Cardioplegic solutions that are used during CPB for myocardial protection do not prevent the injury that occurs after reperfusion.[4, 6, 7] Moreover, the administration of these solutions themselves has been shown to cause some degree of endothelial dysfunction.[1, 2] The detection of endothelial dysfunction in the setting of cardioplegic arrest is difficult and relies on microvascular preparations, isolated heart models, or indirect serologic indices of injury.

Initial observations with MCE in our laboratory suggested that myocardial opacification caused by sonicated albumin microbubbles persisted for a long duration during infusion of crystalloid cardioplegic solution.[16, 17] Normally, these bubbles possess similar rheologic properties to those of RBCs and pass unencumbered through the microcirculation.[19, 20] In the current study, the mean microbubble transit rate was abnormally prolonged during crystalloid cardioplegic solution administration, yet the RBC tran-

Table I. *Variables measured during administration of different cardioplegic perfusates in 12 dogs*

	Crystalloid cardioplegic solution	*Arterial blood*	*Venous blood*	*p Value*
Aortic root pressure (mm Hg)	54 ± 15	46 ± 10	45 ± 13	0.16
Coronary vascular resistance (dynes · sec · cm^{-5})*	26,412 ± 9266	22,070 ± 7054	21,708 ± 6547	0.25
K^+ (mEq · L^{-1})	24.4 ± 3.1	24.2 ± 6.2	26.8 ± 2.3	0.13
Osmolality (mOsm · kg^{-1})	315 ± 12	320 ± 10	327 ± 15	0.10
pH	7.49 ± 0.22	7.54 ± 0.08	7.50 ± 0.06	0.07
P_{O_2} (mm Hg)	158 ± 75	386 ± 110	43 ± 13	<0.001
P_{CO_2} (mm Hg)	15 ± 5	14 ± 2	17 ± 3	0.12
Temperature (°C)	22 ± 12	25 ± 11	25 ± 11	0.80

*Calculated by the formula (aortic pressure)(1332 dynes · cm^{-2} · mm Hg)(60 sec · min^{-1})/(infusion rate).

Table II. *Demographic and clinical data in patients receiving arterial-venous or venous-arterial reperfusion*

	Arterial-venous (n = 6)	*Venous-arterial (n = 6)*	*p Value*
Age (median, yr)	68	64	0.08
Weight (kg)	77 ± 18	85 ± 14	0.39
Body surface area (m^2)	1.9 ± 0.2	2.0 ± 0.1	0.27
Cardioplegic solution dose (ml)*	1975 ± 189	1997 ± 316	0.92
CPB time (min)	97 ± 17	78 ± 21	0.11
Aortic crossclamp time (min)	62 ± 8	49 ± 11	0.03
Bypassed vessels (median, n)	3.5	3.0	0.29

*(Blood cardioplegic solution flow rate) × (total time of cardioplegic solution administered).

Table III. *Analysis of arterial and venous perfusates in patients during reperfusion*

	Arterial (n = 12)	*Venous (n = 12)*	*p Value*
Hematocrit (%)	18.0 ± 4.3	15.8 ± 3.3	0.07
Potassium concentration (mEq · L^{-1})	9.9 ± 2.7	7.3 ± 3.6	0.08
pH	7.31 ± 0.03	7.14 ± 0.08	<0.001
P_{O_2} (mm Hg)	151 ± 38	48 ± 4	<0.001
P_{CO_2} (mm Hg)	39 ± 3	44 ± 11	0.10

sit rate remained normal, indicating that these bubbles might either interact with the myocardial microvasculature in this setting or rely on blood to facilitate their circulatory transit. The former hypothesis is supported by previous intravital microscopic observations that some of the albumin microbubbles injected intravascularly adhere to venular endothelium after cardioplegic solution delivery and reperfusion.[21] Instead of behaving like RBCs, these bubbles behaved like leukocytes, which were also seen adhering to the endothelium as a consequence of endothelial injury.[21] We believe that endothelial adherence of the microbubbles in response to cardioplegia-induced injury resulted in a prolonged myocardial microbubble transit rate in our study. We have previously shown that adherence of only a relatively small proportion of the albumin microbubbles injected is needed to produce the persistent myocardial opacification.[25] Physical entrapment of the microbubbles, caused by changes in microcirculatory dimension, can be excluded as a reason for persistent myocardial opacification because both the RBC transit rates and coronary vascular resistance values were similar between perfusate groups.

Role of blood during cardioplegic arrest. Most of the injury after cardioplegic arrest is thought to occur after reperfusion[6, 7] and results from the release of multiple cytotoxic factors. Oxygen-derived free radicals are one of many leukocyte-derived chemical mediators of endothelial injury.[11] Because injury resulting in endothelial dysfunction may occur early after reperfusion,[9, 10] sources of reactive oxygen intermediates other than leukocytes may be more important; these include calcium-dependent xanthine oxidase production of O_2^- and H_2O_2[26] and generation of peroxynitrite from excess nitric oxide production.[27] The microcirculatory endothelium is extremely sensitive to these compounds and functional abnormalities are seen very early after exposure to oxygen free radicals.[9-11]

The use of blood cardioplegia has been shown to preserve endothelial integrity and function[5, 13] rather than to cause the deleterious effects that are seen after sudden reperfusion. The beneficial effect of blood may be independent of its oxygen-carrying capacity because of a leftward shift of the oxygen-hemoglobin dissociation curve.[28] Only dissolved rather than hemoglobin-bound oxygen is delivered to the tissue. That the P_{O_2} level of the perfusate in

the animal protocol had no relation to the microbubble transit rate in our study also suggests that the beneficial effect of blood may be independent of oxygen delivery. Furthermore, when results of reperfusion with only arterial and venous blood were compared in patients, we found an inverse correlation between Po_2 values and the microbubble transit rate during reperfusion.

The beneficial effect of blood in cardioplegic solution may instead be a result of its action as a scavenger of oxygen-derived free radicals.[13] The addition to cardioplegic solutions of other scavenger compounds and inhibitors of free radical production has been observed to have similar effects.[7, 9, 10] The lower oxygen content and possibly better free-radical scavenging properties of venous blood during cardioplegia may have produced less endothelial injury and, hence, faster microbubble MCE transit rates. In patients, we found that reperfusion with venous blood resulted in more rapid microbubble transit than reperfusion with superoxygenated arterial blood. That faster transit rates with venous blood are caused by less endothelial injury is commensurate with observations in hypoxic animals in which use of hyperoxic cardioplegic or reperfusion solutions resulted in oxidative injury.[14, 15] The limitation of oxygen delivery by use of venous blood has also been shown to reduce free radical–mediated reperfusion injury in the rat intestine.[29] Hypoxic reperfusion of isolated skeletal muscles after ischemia has been shown to attenuate the increase in vascular resistance and permeability,[30] thereby supporting the theory that endothelial injury can be lessened with low Po_2 reperfusion. The finding that venous blood was less beneficial when its use was preceded by use of arterial blood implies that reperfusion damage may be cumulative, although this conclusion is somewhat confounded by the longer crossclamp time in this group.

Study limitations. Although evidence in this and other studies supports the notion that microbubbles adhere to injured endothelium,[21] the mechanism of this interaction remains unclear. Because prolongation of the MCE transit rate during delivery of crystalloid cardioplegic solution can be rapidly reversed, the mechanism probably does not involve severe structural alteration of the endothelium or immediate expression of adhesion molecules. The rapid reversal would, rather, imply a more dynamic process that may involve either the endothelial glycocalyx or preexisting cell surface molecules, which are altered under various pathologic states. Further investigation will be needed to determine the cause of such interactions wherein the causal relationship between oxidative injury and microbubble adherence may be established by the addition of inhibitors of free radical formation and measurement of venous effluent oxidative metabolites such as conjugated dienes.[15] Elucidation of the mechanisms of microbubble adherence to the endothelium would also offer insight into the beneficial properties of blood compared with crystalloid solutions during cardioplegic arrest. Because all patients in the intraoperative portion of the study received both arterial and venous reperfusion, comparison of effects on left ventricular function and on long-term clinical outcomes was not possible but could be investigated in future studies.

Defined normal values do not exist for the myocardial transit rates of microbubbles and RBCs because these parameters are influenced by the input function, which is determined by both the site and rate of injection. We held all of these variables constant, as well as the myocardial blood/cardioplegic solution flow rate, which could also alter microbubble transit rate. The transit rate is also inversely proportional to the volume of distribution within the myocardium[21] and, although we could not independently confirm that myocardial blood volume was constant, similar coronary vascular resistance values during different infusions would suggest that such was the case.

Clinical applications. The evaluation of MCE transit rates has the potential to directly assess endothelial function under various conditions. With this technique, we have demonstrated that the use of venous rather than arterial blood may be advantageous for reperfusion after cardioplegic arrest. These results have an important bearing on the determination of optimal cardioplegic solution composition during coronary artery bypass procedures. Further studies are required to understand the mechanisms of the interaction between microbubbles and the endothelium.

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