CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

EFFECTS OF CARDIOPLEGIA ON VASCULAR FUNCTION AND THE "NO-REFLOW" PHENOMENON AFTER ISCHEMIA AND REPERFUSION: STUDIES IN THE ISOLATED BLOOD-PERFUSED RAT HEART

The ability of cardioplegia to protect against cardiac contractile dysfunction caused by ischemia and reperfusion is well established. The effects of cardioplegia on vascular injury and the no-reflow phenomenon, however, remain controversial. We used the blood-perfused rat heart to study the effect of St. Thomas' Hospital cardioplegic solution on postischemic endothelium-dependent and endothelium-independent vascular function, the extent of the no-reflow phenomenon, and the temporal relationship between postischemic vascular and contractile function. Isolated rat hearts (16 per group) perfused with blood from a support rat at 60 mm Hg were subjected to 10, 20, 30 or 40 minutes of global ischemia and 40 minutes of reperfusion at 37° C. Eight hearts in each group also received cardioplegia (45 mm Hg for 2 minutes) before ischemia. Left ventricular developed pressure was measured with an intraventricular balloon. At the end of reperfusion, a bolus of 250 µg nitro-L-arginine methyl ester was infused to assess endothelium dependent vascular function. After a 20-minute wash-out, 25 µg sodium nitroprusside was infused to assess endothelium-independent vascular function. Fluorescein (1 ml, 1% weight/volume) was then infused to assess no-reflow; this involved freezing the hearts, cutting them into transverse sections (10 × 1 mm), video recording the sections under ultraviolet light, digitizing the images, and analyzing density of fluorescence. No-reflow was defined as a flow of less than 5%. Compared with nonischemic control responses, endothelium-independent vascular function was significantly decreased only after 30 and 40 minutes of ischemia ($48.1\% \pm 3.8\%$ and $24.3\% \pm 7.4\%$, $p < 0.05$), but it was significantly protected by cardioplegia ($66.6\% \pm 3.9\%$ and $64.5\% \pm 5.2\%$, $p < 0.05$). A significant reduction in endothelium-dependent vascular function was observed after 40 minutes of ischemia ($-31.8\% \pm 6.6\%$ vs $-50.4\% \pm 1.6\%$ in control hearts, $p < 0.05$), and again this was improved by cardioplegia ($-45.0\% \pm 3.4\%$, $p < 0.05$ vs ischemic group). Areas of no reflow were present after 30 and 40 minutes of ischemia ($11.9\% \pm 6.8\%$ and $33.4\% \pm 14.1\%$ of left ventricular mass), and at each time period they were significantly decreased by cardioplegia ($0.7\% \pm 0.4\%$ and $3.8\% \pm 1.6\%$, $p < 0.05$). Postischemic contractile dysfunction was observed before any vascu-

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lar alteration was apparent. After only 20 minutes of ischemia, the postischemic recovery of left ventricular developed pressure fell to $56.7\% \pm 4.0\%$, but both endothelium-dependent vascular function and endothelium-independent vascular function were unaffected. In conclusion, vascular alterations are apparent only after prolonged periods of ischemia, longer than those required to observe contractile dysfunction, and cardioplegia protects against postischemic endothelium-dependent and endothelium-independent vascular dysfunction and reduces the extent of the no-reflow phenomenon. (J THORAC CARDIOVASC SURG 1996;111:432-42)

Most studies on the consequences of myocardial ischemia and reperfusion have focused on the vulnerability of the *myocyte* to injury and on the ability of the heart to recover contractile function. Contractile end points have therefore been most commonly used in the development and assessment of cardioplegic solutions for clinical practice. Recently, however, there has been increasing interest in the effects of ischemia and reperfusion on the microvasculature and its function.¹ It is now appreciated that the survival of the heart as a whole may in part depend on the ability of the microcirculation to deliver and distribute blood flow adequately during reperfusion. As a consequence, cardioplegic solutions have been reevaluated in a number of studies to ascertain whether their protective properties extend to the coronary vasculature or whether these solutions may even be damaging to the microcirculation and its function. The first report of a possible damaging effect of cardioplegia on the coronary vasculature was by Carpentier, Murawsky, and Carpentier² in 1981, who demonstrated a cytotoxic effect of various cardioplegic solutions on cultured endothelial cells. Whether cardioplegic solutions can damage the vascular elements in whole hearts is less clear, but it has been proposed that the high potassium concentration of most solutions,^{3,4} and other factors, such as high infusion pressures or flows,⁵⁻⁷ may be deleterious. The elucidation of the effect of chemical cardioplegia per se on the vasculature is of course complicated by the coincidental effects of ischemia on the vascular elements.

In this study, the isolated blood-perfused rat heart preparation was used to investigate the effect of St. Thomas' cardioplegic solution on postischemic endothelium-dependent vascular function (EDVF) and endothelium-independent vascular function (EIVF), the extent of the "no-reflow" phenomenon, and the temporal relationship between postischemic vascular alterations and contractile dysfunction.

Material and methods

Animals. Male Wistar rats weighing 200 to 250 gm (donor rats) and 400 to 450 gm (support rats) were used. All animals received care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Isolated blood-perfused rat heart preparation (Fig. 1). Support rats were anesthetized with pentobarbital (60 mg/kg intraperitoneally) and placed supine on a heating pad ($37.0^\circ \pm 0.5^\circ \text{C}$). The rats were allowed to breathe spontaneously a humidified mixture of 95% oxygen plus 5% carbon dioxide through a Venturi mask. The gas flow rate was set at 350 to 400 ml/min to maintain arterial oxygen and carbon dioxide tensions within the physiologic range (100 to 140 mm Hg and 35 to 45 mm Hg, respectively). The rats received anticoagulation with heparin (1000 IU/kg intravenously), and a femoral artery and vein were cannulated for the arterial blood supply to the donor heart and the return of blood to the support rat. Anesthesia was administered as necessary (10 mg/kg pentobarbital intraperitoneally). The support rats were used for no longer than 5 hours.

Donor rats were anesthetized and given heparin in the same manner as support rats. Hearts were then rapidly excised and perfused in the Langendorff mode with arterial blood delivered from the support rat by a peristaltic pump. Perfusion was controlled with the aid of a pump speed driver that was designed to adjust the flow rate continuously to maintain a constant aortic pressure of 60 mm Hg. Coronary flow was calculated from the pump speed (a nomogram was used to calculate absolute flow from the number of revolutions per minute), which was recorded on chart paper throughout the experiment. The temperature of the blood and the isolated heart was maintained at $37.0^\circ \pm 0.5^\circ \text{C}$ by a thermostatically regulated heat-exchange system. Coronary effluent was collected into a venous reservoir and reinfused by gravity into the support rat through a blood filter (pore size 200 μm). A compliant balloon catheter attached to a pressure transducer was inserted into the left ventricle through the opened left atrial appendage and inflated to achieve a constant left ventricular end-diastolic pressure of 4 mm Hg. Heart rate was maintained at 380 beats/min by right atrial pacing.

Fig. 1. Diagrammatic representation of the isolated blood-perfused rat heart preparation. *LV*, left ventricular.

Protocol. Hearts (16 per group) were perfused aerobically for 20 minutes to allow stabilization of temperature, heart rate, left ventricular end-diastolic pressure, left ventricular developed pressure (LVDP), and coronary flow and then randomly subjected to 10, 20, 30 or 40 minutes (groups 1, 2, 3, and 4, respectively) of normothermic global ischemia and 40 minutes of reperfusion. Eight hearts in each group also received St Thomas' Hospital cardioplegic solution number 2 (110.0 mmol/L sodium chloride, 16.0 mmol/L potassium chloride, 16.0 mmol/L magnesium chloride, 1.2 mmol/L calcium chloride, and 10.0 mmol/L sodium hydrogen carbonate) for 2 minutes at a perfusion pressure of 45 mm Hg. The postischemic recoveries of LVDP and coronary flow were continuously recorded. At the end of reperfusion, a 250 μ g (3 mmol/L) bolus of nitro-L-arginine methyl ester (L-NAME) was infused to assess EDVF. After a 20-minute washout, 25 μ g (0.3 mmol/L) sodium nitroprusside (SNP) was infused to assess EIVF. This sequence of drug administration was deliberately chosen because of the long-lasting effects of

L-NAME and the slow recovery of flow that occurs after its administration. This pattern contrasts with the shorter-lasting effects of SNP. During the assessment of vascular function, the hearts were unloaded by deflation of the intraventricular balloon. This was undertaken to minimize any possible changes in coronary flow during drug infusion that might occur in response to any changes in left ventricular work. Fluorescein (1 ml, 1% weight/volume) was infused 20 minutes after completion of the assessment of vascular reactivity. Hearts were then frozen to allow assessment of the no-reflow state. A time-matched aerobic control group of hearts ($n = 8$) was aerobically perfused to obtain control vascular responses to L-NAME and SNP and the normal pattern of flow distribution.

Preparation and infusion of drugs. L-NAME and SNP were obtained from Sigma Chemical Co. (Poole, United Kingdom). Drugs were dissolved in deionized water to obtain standard concentrations of 500 μ g/ml for L-NAME and 50 μ g/ml for SNP. Aliquots of 0.5 ml were drawn into 1 ml syringes. The solutions were frozen in the syringes

with liquid nitrogen and were then lyophilized for 24 hours and stored at -30°C until use. Immediately before use, the drugs were redissolved in a fixed volume (0.7 ml) of arterial blood taken from the support animal; they were then infused into the isolated perfused heart. Because coronary flow varies, the same dose of drug was infused at different rates in different hearts to achieve the required blood concentration. This procedure was facilitated by the introduction of an infusion line in parallel with the main perfusion line. In this way, any changes in perfusion characteristics caused by alterations in blood volume, hematocrit, or viscosity were avoided (preliminary studies had shown that the coronary vasculature reacts to the infusion of volumes of normal saline solution as small as 0.1 ml). During drug infusion, the coronary effluent was discarded and replaced with an equivalent quantity of fresh homologous blood to avoid any possible drug effect on the support rat.

Assessment of flow distribution and calibration. At the end of the experimental protocol, hearts were switched to crystalloid Langendorff perfusion (bicarbonate buffer at 60 mm Hg and 37°C) for 1 to 2 minutes to wash out blood from the coronary circulation. A bolus of fluorescein solution (1 ml, 1% weight/volume; Sigma Chemical) diluted in bicarbonate buffer was then infused (60 mm Hg and 37°C) to delineate the distribution of flow. Hearts were then frozen and cut into 10 transverse sections 1 mm thick. The right ventricle was separated from the rest of the heart. The surface fluorescein of the sections was then video recorded with a CCD camera (Pulnix America, Inc., Basingstoke, United Kingdom) in a dark room under standard conditions of illumination (long-wave ultraviolet light) and at a fixed focal distance and magnification with a macro-zoom lens (18 to 108 mm, f2.5 macro-zoom lens; RS Components Ltd., Corby, United Kingdom). Analog images were then digitized and analyzed for fluorescence density (NIH Image software, Twilight Clone BBS, Silver Spring, Md.). Calibration of the system was achieved by infusing decreasing concentration of fluorescein into a series of aerobically perfused hearts to obtain a correlation between density of tissue fluorescence and amount of infused fluorescein (Fig. 2, A). Gray-scale density (GSD), which ranges from 0 (absolute white) to 256 (absolute black), was used to measure the intensity of fluorescence, which was arbitrarily divided into three bands. Areas with GSDs less than 149 (amount of fluorescein $>50\%$) were classified as having "good flow." Areas with GSDs of 150 to 170 (amount of fluorescein 50% to 5%) were classified as having "low flow." Areas with GSDs greater than 171 (amount of fluorescein $<5\%$) were classified as having "no flow." Fig. 2 (B) shows the typical flow distribution of aerobic control hearts and hearts subjected to 40 minutes of ischemia and 40 minutes of reperfusion.

Inclusion criteria. After instrumentation and 15 minutes of aerobic perfusion, only isolated hearts with LVDP greater than 130 mm Hg and coronary flow between 1.7 and 2.5 ml/min were entered in the study. Any excluded hearts were replaced immediately.

Expression of results and statistical analysis. LVDP was calculated by subtracting left ventricular end-diastolic pressure from left ventricular systolic pressure. Posts ischemic recovery of LVDP was expressed as a percentage of

the aerobic control value. Vascular responses after the administration of L-NAME and SNP were calculated as percentage changes of the baseline flow according to the following equation:

$$\% \text{ change in CF} = (rCF \times 100/iCF) - 100$$

where CF is coronary flow, iCF is initial coronary flow (before drug infusion), and rCF is the coronary flow in response to drug infusion. For the assessment of tissue distribution of flow, the pixels with the same GSD present in the 10 images obtained from a single heart were added and then grouped in three GSD bands (1 to 149, 150 to 170, and 171 to 256). The amounts of good flow, low flow, and no flow, corresponding to the number of pixels in each band, were expressed as percentage values of the total number of pixels in the heart. All results were reported as mean values $\pm$ standard error of the mean. Correlation between amount of fluorescein (percentage of control) and density of fluorescence (in GSDs) was carried out by linear regression analysis. Statistical evaluation of data was performed with analysis of variance. When the analysis of variance showed statistical significance ($p \leq 0.05$), the Student's t test with Bonferroni's correction was applied.

Results

Seven hearts were excluded from the study: five because of concomitant poor coronary flow and LVDP, two because of poor coronary flow alone, and one for excessive coronary flow. The aerobic control mean values for LVDP and coronary flow for all hearts in the study were 168 ± 3 mm Hg and 2.1 ± 0.1 ml/min, respectively, and there were no significant differences between groups.

Posts ischemic recovery of LVDP. As shown in Fig. 3 (A), the posts ischemic recovery of LVDP was little affected with mild (10-minute) ischemia. Prolonged periods of ischemia resulted in a progressive decline in recovery, however, so that after 40 minutes of ischemia recovery of LVDP was less than 15% of the preischemic aerobic control values. As expected, cardioplegia improved the recovery of LVDP in all instances.

Posts ischemic recovery of coronary flow. As shown in Fig. 3 (B), the posts ischemic recovery of coronary flow was not impaired by ischemic periods shorter than 20 minutes, a time at which the recovery of LVDP was already reduced by 50%. Recovery of coronary flow was only impaired after more severe ischemia, with recovery falling to 60% and 24% after 30 and 40 minutes of ischemia, respectively. As observed with LVDP, cardioplegia also improved the posts ischemic recovery of coronary flow to 95% and 65% of their preischemic control values after 30 and 40 minutes of ischemia, respec-

Fig. 2. **A,** Correlation between the amount of fluorescein in the tissue and the intensity of light emitted (measured as GSD). *Dotted lines* represent 95% confidence limits. **B,** Distribution of pixels in control aerobic hearts and hearts that had been subjected to ischemia (40 minutes) and reperfusion (40 minutes). GSD has been divided into three bands: good flow (<149), low flow (150 to 170), and no reflow (>256). *SD*, standard deviation.

Fig. 3. Recovery of LVDP (A) and coronary flow (B), expressed as percentages of preischemic control values in the ischemia-only group (black bars) and cardioplegia group (cross-hatched bars) at different times of ischemia. Asterisk indicates $p < 0.05$ between ischemia-alone group and corresponding cardioplegia group.

tively ($p < 0.05$ vs corresponding ischemia-alone group).

Postischemic recovery of vascular function. As shown in Fig. 4, the vascular responses to SNP and L-NAME in time-matched aerobic control hearts were $96.5\% \pm 7.0\%$ and $-50.4\% \pm 1.6\%$, respectively. As reflected by coronary flow, both EIVF and EDVF were not significantly affected by ischemic periods of 20 minutes or shorter. Extending the time of ischemia to more than 20 minutes, however, resulted in progressive vascular dysfunction with concomitant deterioration of EIVF and EDVF. Responses to SNP were reduced to $48.1\% \pm 3.8\%$ and $24.3\% \pm 7.4\%$, and responses to L-NAME were

Fig. 4. Vascular responses to SNP (A) and L-NAME (B), expressed as percentage changes from baseline coronary flow (flow immediately before drug infusion) in time-matched aerobic control hearts (open bars), ischemia-only group (black bars), and cardioplegia group (cross-hatched bars) after different durations of ischemia. Asterisk indicates $p < 0.05$ between ischemia-alone group and corresponding cardioplegia group. Dagger indicates $p < 0.05$ versus nonischemic control group.

reduced to $-36.3\% \pm 3.7\%$ and $-31.8\% \pm 6.6\%$ after 30 and 40 minutes of ischemia, respectively. The flow changes induced by SNP were in general more profound than those observed with L-NAME, both with and without cardioplegia. This difference attained statistical significance in the groups subjected to 40 minutes of ischemia ($p < 0.05$). Interestingly, cardioplegia significantly improved EIVF and EDVF in all instances ($66.6\% \pm 3.9\%$ and $64.5\% \pm 5.2\%$ for SNP and $-47.6\% \pm 1.2\%$ and

Fig. 5. Areas of good flow (A), low flow (B), and no reflow (C) expressed as percentages of the left ventricle analyzed in the ischemia-only group (black bars) and cardioplegia group (cross-hatched bars) at different times of ischemia. Asterisk indicates $p < 0.05$ between ischemia-alone group and corresponding cardioplegia group.

$-45.0\% \pm 3.4\%$ for L-NAME after 30 and 40 minutes of ischemia; $p < 0.05$ for all values).

Postischemic myocardial distribution of flow. As shown in Fig. 5, the myocardial distribution of flow

was unaffected after ischemic periods of 30 minutes or shorter, a time at which the postischemic recovery of LVDP and coronary flow and vascular reactivity were already significantly impaired. The distribution of flow was significantly impaired after 40 minutes of ischemia, however; $33\% \pm 14\%$ of the left ventricle exhibited areas of no-reflow. Cardioplegia significantly reduced the no-reflow areas to $4\% \pm 2\%$ ($p < 0.05$). Fig. 6 shows that no-reflow areas were located predominantly in the midmyocardium and endocardium and that this phenomenon was prevented to a large extent by cardioplegia.

Discussion

This study demonstrated that the contractile function of the myocyte is more susceptible to ischemic injury than is the reactivity of the vasculature to vasoactive agents and that cardioplegia protects against postischemic vascular dysfunction and improves coronary vascular patency. These findings and their possible clinical implications are discussed further.

Importance of the experimental preparation and the technique for the assessment of EDVF and EIVF. The effects of ischemia and reperfusion on the coronary vasculature have been investigated extensively with a wide variety of experimental preparations, ranging from coronary artery rings to whole-heart preparations, both in vitro and in vivo. Each of these preparations has certain advantages and disadvantages. For example, vascular rings offer the convenience of an isolated preparation without the influence of neurohumoral factors or the effect of other tissue cell types (e.g., myocytes). Vascular rings are easily damaged during preparation, however, and administered agents can reach the vessel by both luminal and abluminal pathways. In addition, vascular rings cannot provide information on the microvasculature. In vivo whole-heart preparations do not have these inconveniences of vascular rings, but in these preparations the study of vascular reactivity is more difficult. Another approach to the study of vascular function is the use of isolated hearts; however, the common use of crystalloid solutions in these preparations causes unphysiologically high coronary flows and vascular permeability alterations that may impair vascular reactivity during aerobic conditions. To overcome these problems, we used the isolated rat heart perfused with whole blood from a support rat. In this preparation, the heart is perfused with autologous blood and the perfusion pressure can be tightly controlled. It

Fig. 6. Representative digitized images of the left ventricle. The two images are a control heart (A) and a heart subjected to 40 minutes of ischemia and 40 minutes of reperfusion (B). Similar areas taken from the control heart and from the ischemic and reperfused heart are enlarged and displayed separately as a graphic distribution of GSDs of the image pixels. The displayed GSD was divided into three bands.

should be conceded that, although denervated, the isolated heart can be influenced by the depth of anesthesia of the support rat. This is, however, a situation that can be readily controlled so that heart rate and mean arterial pressure (data not shown) or the plasma levels of catecholamines (Lawson C, personal communication) in the support rat are not altered during the 5-hour experimental time. To separate the effects of ischemia and reperfusion from those of cardioplegia on the endothelium and smooth muscle of the vasculature, we used agents acting selectively on each of these elements. The nitric oxide donor SNP was used for the measurement of the EIVF. For the study of EDVF, the nitric oxide synthase inhibitor L-NAME was used. This choice was based on the consistently good responses

obtained with this intervention in previous studies⁸ that used a preparation identical to the one in this study. In addition, our earlier studies showed that the use of nitric oxide synthase stimulators (such as acetylcholine, serotonin, and adenosine triphosphate) afford smaller but less predictable responses. These results are supported by the findings of species differences in the distribution and activation of receptor subtypes.⁹⁻¹²

The relative susceptibilities of the myocyte and the coronary vasculature to ischemic injury. The precise temporal relationship between myocardial and vascular dysfunction induced by ischemia has not been clearly characterized, and to our knowledge this is the first study to investigate simultaneously the effects of increasing durations of ischemia on con-

tractile function, coronary flow, coronary responsiveness, and vascular patency. Our results indicate that the myocyte is more susceptible to ischemic injury than is the vasculature, and the vascular smooth muscle is in turn more susceptible than is the endothelium. Kloner, Ganote, and Jennings¹³ studied dogs subjected to 40 and 90 minutes of regional ischemia and 20 minutes of reperfusion, and suggest that the presence of no-reflow areas was caused by myocardial necrosis. In these studies, however, the assessment of tissue injury was carried out by ultrastructural techniques, and the question of which tissue component—the myocyte or the vasculature—was responsible for initiation of the no-reflow phenomenon could not be answered. In this connection, Dauber and coworkers¹⁴ observed in anesthetized dogs subjected to 0, 15, 30 and 60 minutes of regional ischemia and reperfusion that functional abnormalities may precede morphologic changes. Contractile dysfunction was not reported in that study. In discussing relative patterns of injury and recovery, it is important to distinguish between vulnerability to injury and the ease with which tissue recovers from injury. The fact that contractile function is more rapidly injured than vascular function does not mean that its recovery is necessarily faster. In this connection, Kim and associates¹⁵ showed in the dog heart that, after 15 minutes of regional ischemia followed by 120 minutes of reperfusion, vascular dysfunction returned to normal completely within 90 minutes of reperfusion, whereas contractility was still depressed after 120 minutes of reperfusion.

In our experiments, reduction in coronary flow occurred concurrently with alterations in EIVF rather than in EDVF, suggesting that injury to the vascular smooth muscle, rather than the endothelium, may account for the overall reduction in vascular responsiveness and increase in vascular resistance. Both vascular and contractile dysfunction preceded the onset of the no-reflow phenomenon. These results are supported by those of other investigators showing increased vascular resistance and impaired coronary vascular reactivity after mild to moderate ischemia.¹⁶⁻¹⁸

The effect of cardioplegia on the postischemic recovery of vascular reactivity. In a previous study from our laboratory,¹⁹ in which the isolated rat mesentery was perfused with a crystalloid solution and then subjected to ischemia, it was shown that cardioplegia administered as a single dose neither protected nor injured the vasculature. Multiple in-

fusions (every 30 minutes) of cardioplegia, however, conferred some protection on both endothelium and vascular smooth muscle. In these studies, in which the isolated rat heart was perfused with blood, single-dose cardioplegia resulted in a significant protection of endothelial and vascular smooth muscle reactivity. The cardioplegia-induced protection of vascular reactivity was paralleled by concomitant protection of coronary flow and contractile function, and it also resulted in an amelioration of the no-reflow phenomenon. These results are supported by another study from our laboratory,²⁰ in which both single-dose and multidose cardioplegia afforded protection to the vasculature of the immature pig heart perfused with blood.

In our studies, we used St. Thomas' Hospital cardioplegic solution. We did not explore the influences of the individual components of the solution or of its mode of administration. It may therefore be possible that changing the composition of the cardioplegic formulation (e.g., potassium or magnesium concentration) or administering the solution in a different way from the one used in our studies may have different or even detrimental effects on the postischemic recovery of vascular reactivity.

Possible clinical implications. This study shows that the St. Thomas' cardioplegic solution has no detrimental effects on the coronary vasculature of the rat heart. On the contrary, the solution affords protection of both contractile and vascular function. Although these findings are necessarily limited by their observation in the rat heart, they do suggest that there may be scope for investigating whether solutions could be designed and optimized for the protection of the vasculature as well as the myocyte.

REFERENCES

1. Hearse DJ, Maxwell L, Saldanha C, Gavin JB. The myocardial vasculature during ischemia and reperfusion: a target for injury and protection. *J Mol Cell Cardiol* 1993;25:759-800.
2. Carpentier S, Murawsky M, Carpentier A. Cytotoxicity of cardioplegic solutions: evaluation by tissue culture. *Circulation* 1981;64(2 Pt2):90-5.
3. Saldanha C, Hearse D. Coronary vascular responsiveness to 5-hydroxytryptamine before and after infusion of hyperkalemic crystalloid cardioplegic solution in the rat heart. *J THORAC CARDIOVASC SURG* 1989;98:783-7.
4. Mankad PS, Chester AH, Yacoub MH. Role of potassium concentration in cardioplegic solutions in

- mediating endothelial damage. *Ann Thorac Surg* 1991;51:89-93.
5. Molina JE, Galliani CA, Einzig S, Bianco R, Rasmussen T, Clack R. Physical and mechanical effects of cardioplegic injection on flow distribution and myocardial damage in hearts with normal coronary arteries. *J THORAC CARDIOVASC SURG* 1989;97:870-7.
6. Evora PR, Pearson PJ, Schaff HV. Crystalloid cardioplegia and hypothermia do not impair endothelium-dependent relaxation or damage vascular smooth muscle of epicardial coronary arteries. *J THORAC CARDIOVASC SURG* 1992;104:1365-74.
7. Cartier R, Pellerin M, Hollmann C, Pelletier LC. Effects of pressure and duration of hyperkalemic infusions on endothelial function. *Ann Thorac Surg* 1993;55:700-5.
8. Argano V, Galinanes M, Edmondson SJ, Hearse DJ. A novel approach for assessing endothelium-dependent vascular function in the isolated blood-perfused rat heart [Abstract 443]. *J Mol Cell Cardiol* 1994;26:106.
9. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991;43:109-42.
10. De Mey JG, Vanhoutte PM. Heterogeneous behaviour of the canine arterial and venous wall. Importance of the endothelium. *Circ Res* 1982;51:439-47.
11. Hollenberg NK. Serotonin and vascular responses. *Annu Rev Pharmacol Toxicol* 1988;28:41-59.
12. Nyborg NC, Mikkelsen EO. 5-Hydroxytryptamine does not release endothelium-derived relaxing factor in rat isolated coronary arteries. *Eur J Pharmacol* 1990;186:295-300.
13. Kloner RA, Ganote CE, Jennings RB. The no-reflow phenomenon after temporary coronary occlusion in dogs. *J Clin Invest* 1974;54:1496-508.
14. Dauber IM, Van Benthuyzen KM, McMurtry IF, et al. Functional coronary microvascular injury evident as increased permeability due to brief ischemia and reperfusion. *Circ Res* 1990;66:986-98.
15. Kim YD, Fomsgaard JS, Heim KF, et al. Brief ischemia-reperfusion induces stunning of endothelium in canine coronary artery. *Circulation* 1992;85:1473-82.
16. Nicklas JM, Gips SJ. Decreased coronary flow reserve after transient myocardial ischemia in dogs. *J Am Coll Cardiol* 1989;1:195-9.
17. Vanhaecke J, Flameng W, Borgens M, Jang IK, Van-de-Werf F, De Geest H. Evidence for decreased coronary flow reserve in viable post-ischemic myocardium. *Circ Res* 1990;67:1201-10.
18. Bolli R, Triana JF, Jeroudi MO. Prolonged impairment of coronary vasodilation after reversible ischemia: evidence for microvascular stunning. *Circ Res* 1990;67:332-43.
19. Saldanha C, Hearse DJ. Cardioplegia and vascular injury: dissociation of the effects of ischemia from those of the cardioplegic solution. *J THORAC CARDIOVASC SURG* 1994;108:279-90.
20. Galiñanes M, Saldanha C, Kato H, Elliot MJ, de Leval MR, Hearse DJ. Vascular and contractile function and tissue metabolites after prolonged hypothermic ischaemia and reperfusion: comparison of single-versus multi-dose infusions with two cardioplegic solutions in blood-perfused neonatal pig hearts. *J Mol Cell Biol* 1995;27:1915-30.

Discussion

Mr. Magdi Yacoub (London, United Kingdom). I congratulate you on an extremely elegant study that used a blood-perfused isolated rat heart to examine several issues relating to vascular reactivity during reperfusion.

I have several questions. First, although the blood-perfused isolated model you have used mimics physiologic conditions, how do you explain the extremely low coronary flow observed in your control animals when compared, for example, to crystalloid-perfused rat hearts, in which there is at least an eightfold to tenfold increase in flow? Do you think this is physiologic, or do you think that there is endothelial injury or generation of vasoactive peptides inherent in your model because of the use of a pumping apparatus?

The second question relates to measurements of systolic function. Do you think that measurement of generated pressure at a predetermined end-diastolic volume is enough to quantify mechanical function in an isovolumic model, or it would be more useful to use a pressure-volume relationship, as suggested by Webber and Geneke, particularly when the conditions of the heart will differ markedly before and after reperfusion?

Third, how do you explain the vulnerability of the smooth muscle cell compared with the endothelial cell? Previous studies have suggested that endothelial cells are a lot more sensitive than smooth muscle cells.

My final, possibly trivial, point is that when the animals are syngeneic have you excluded any immunologic effect? Some recent studies have shown that allogeneic or xenogeneic polymorphonuclear leukocytes are highly active against endothelial cells.

Dr. Argano. In answer to your first question, we have made an effort to perfuse the isolated rat hearts with blood rather than a crystalloid solution because of the physiologic relevance of blood elements and vessel wall interactions. We have found from previous studies that the average coronary flow of the blood-perfused rat heart reflects more closely the coronary flow of the rat heart in vivo. We have also observed a stable level of catecholamine levels for at least 5 hours of reperfusion. Although the high coronary flow in the crystalloid-perfused rat heart may be caused by the lower viscosity of the perfusion fluid, we have also observed a lower coronary vasodilator reserve, which may suggest the presence of increased vasodilator tone in the crystalloid-perfused preparation.

In answer to your second question, we did use pressure-volume curves to assess systolic and diastolic function of all hearts. These were not shown today for the sake of

time and clarity and because they correlated well with the recovery of LVDP.

In reply to your third question, we were surprised to see that vascular smooth muscle was somewhat more affected than the endothelium. Similar findings were obtained recently by Saldanha and Hearse in the isolated rat mesentery and published in *THE JOURNAL OF THORACIC AND CARDIOVASCULAR SURGERY*.

To answer your final question, the animals were all syngeneic and we would not expect any immunologic effects.

Dr. Franklin Lawrence Rosenfeldt (*Melbourne, Australia*). My question centers around the ability of this preparation to allow you to separate out the individual protective effects of the cardioplegia on the myocardium from those on the coronary vasculature. Did 40 minutes of normothermic global ischemia result in a degree of ischemic contracture? If so, could the protective effect of the cardioplegia be acting more on the myocardium to prevent mechanical compression of the coronary circulation than on the coronary vasculature directly?

The classic way to study the integrity of the vasculature is by means of vessel rings in the organ bath. This allows

one to study the effect of constrictors and dilators on the coronary vasculature itself. This technique can be miniaturized with a myograph, as shown by Mulvany and others (Mulvany MJ, Halpern W. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ Res* 1997;41:19-26.), to work on very small blood vessels.

Therefore, what proportion of the protective effect was myocardial and what was truly vascular protection?

Dr. Argano. I entirely agree with you that the study of vascular rings offers the convenience of an isolated preparation free from interference by other cell types, but it must be conceded that such preparations are not usually perfused with blood, that the isolation procedure is lengthy and may cause smooth muscle or endothelial damage, that drugs can reach the preparation from both the luminal and abluminal pathways, and that the vessels are usually arterial vessels rather than microvessels. Even with the Mulvaney myograph, a particularly demanding instrument used in our Physiology Department, I have rarely seen a vessel smaller than 400 μm mounted.

Dr. Rosenfeldt. With the Mulvaney myograph, you can actually study the microvessels.

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