Differential effects on the mesenteric microcirculatory response to vasopressin and phenylephrine after cardiopulmonary bypass

Tanveer A. Khan, MD, Cesario Bianchi, MD, PhD, Marc Ruel, MD, MPH, Jun Feng, MD, PhD, and Frank W. Sellke, MD

Objective: Mesenteric ischemia is a rare but potentially devastating complication of cardiac surgery with cardiopulmonary bypass. We hypothesized that alterations in mitogen-activated protein kinase pathways contribute to mesenteric microcirculatory dysfunction resulting from cardiopulmonary bypass.

Methods: Pigs underwent cardiopulmonary bypass ($n = 6$) for 90 minutes and postbypass reperfusion for 180 minutes. Sham operations ($n = 6$) were performed on controls. Mesenteric tissue was harvested before bypass and after postbypass reperfusion. Microvascular contraction to phenylephrine and vasopressin was examined by videomicroscopy. Contractile responses with inhibition of the extracellular regulated kinase 1/2 (ERK1/2) pathway by PD98059 ($30 \mu\text{mol/L}$) and p38 kinase inhibition by SB203580 ($1 \mu\text{mol/L}$) also were determined. Activated forms of ERK1/2 and p38 kinase were measured by Western blot. ERK1/2 and p38 activity were localized in mesenteric tissue by immunohistochemistry.

Results: Contractile responses to phenylephrine were increased at 180 minutes after cardiopulmonary bypass ($+49.7\% \pm 5.5\%$, $P < .01$), whereas contraction to vasopressin was unchanged. ERK1/2 pathway inhibition reduced contractile responses to phenylephrine at baseline and 180 minutes after bypass (both $P < .01$) but had no effect on contraction to vasopressin. p38 Kinase inhibition decreased the contractile responses to vasopressin at baseline and 180 minutes after bypass (both $P < .01$) but did not alter the contractile response to phenylephrine. Activated ERK1/2 levels were increased by more than 40% at 180 minutes after bypass ($P < .01$). Protein levels of activated p38 kinase were not changed. The increased ERK1/2 activity was associated with mesenteric arterioles by immunohistochemistry.

Conclusions: A differential pattern of mesenteric vasomotor regulation exists after cardiopulmonary bypass that may contribute to the risk of mesenteric ischemia after cardiac surgery.

From the Division of Cardiothoracic Surgery, Department of Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Mass.

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Address for reprints: Frank W. Sellke, MD, Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, 110 Francis St, LMOB 2A, Boston, MA 02215 (E-mail: fsellke@caregroup.harvard.edu).

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Mesenteric ischemia is a rare but potentially devastating complication of cardiac surgery with cardiopulmonary bypass (CPB). Although mesenteric ischemia occurs in fewer than 1% of cardiac surgical procedures, the mortality rate exceeds 50% in most reported series.^{1,2} Previously, we³ have shown in a porcine model of CPB that mesenteric arterioles have an increased contractile response to phenylephrine after CPB, suggesting a potential mechanism contributing to decreased

Abbreviations and Acronyms

CPB = cardiopulmonary bypass
 ERK1/2 = extracellular regulated kinase 1/2
 MAPK = mitogen-activated protein kinase

perfusion and mesenteric ischemia that complicates cardiac surgery. Alterations in vasomotor regulation involve protein kinase pathways including the mitogen-activated protein kinase (MAPK) pathways. MAPK are serine-threonine protein kinases that are involved in vasomotor function as well as vascular permeability, cytokine production, and reperfusion injury. Three major MAPK families have been identified as the extracellular signal-regulated kinases 1/2 (ERK1/2), c-Jun NH(2)-terminal protein kinases, and p38 kinases, which have established roles in cardiovascular signal transduction cascades.⁴ In a previous study of patients undergoing cardiac surgery, we⁵ demonstrated that CPB resulted in decreased contraction of peripheral, skeletal muscle arterioles to phenylephrine and vasopressin. The impaired contractile responses of peripheral microvessels to phenylephrine were shown to be due to diminished ERK1/2 activity, whereas reduced p38 activity was associated with attenuated contraction to vasopressin.⁵ Thus, vasopressors used to treat hypotension after cardiac surgery, such as phenylephrine and vasopressin, appear to involve different biochemical pathways that regulate the vasomotor function of microvessels. In addition, these studies provided evidence that there are specific vasomotor responses to CPB in each organ system, and that these changes in vascular reactivity may be mediated by alterations in MAPK pathways. We hypothesized that CPB increases the mesenteric vasomotor contrac-

Figure 1. Mesenteric contraction to phenylephrine is increased after CPB. Arterioles from the mesenteric system showed increased contraction to phenylephrine after CPB compared with baseline ($+49.7\% \pm 5.5\%$; $*P < .01$). After sham procedures, contractile responses to phenylephrine were reduced versus the baseline ($-37.0\% \pm 5.1\%$; $*P < .01$).

tile response to vasopressors through alterations in MAPK pathways.

Materials and Methods

Animals and Experimental Design

Animals were housed individually and provided with laboratory chow and water ad libitum. All experiments were approved by the Beth Israel Deaconess Medical Center Animal Care and Use Committee (Institutional Animal Care and Use Committee).

Pigs (35–40 kg) were divided randomly into sham ($n = 6$) and CPB ($n = 6$) groups. Animals in the CPB group underwent CPB for 90 minutes. After 5 minutes of CPB, the aorta was cross-clamped and cold potassium cardioplegic solution was administered for 80 minutes of cardioplegic arrest. The crossclamp then was removed, and the myocardium was reperfused for 5 minutes on CPB, followed by a period of post-CPB reperfusion for 180 minutes. The sham group was subjected to sternotomy and heparinization only.

Surgical Procedure

Pigs were anesthetized with intramuscular ketamine hydrochloride (20 mg/kg) and isoflurane gas by endotracheal intubation and mechanical ventilation. Arterial pressure, heart rate, electrocardiogram, oxygen saturation, arterial blood gases, and temperature

Figure 2. Contractile responses of mesenteric arterioles to phenylephrine are in part dependent on ERK1/2 activity at baseline. Blockade of the ERK1/2 pathway with PD98059 reduced mesenteric microvessel contraction to phenylephrine at baseline in both the sham and CPB groups ($-31\% \pm 4.1\%$ and $-52\% \pm 9.1\%$, respectively; $*P < .01$). Blockade of the p38 pathway with SB203580 had no effect on phenylephrine-induced contraction in either the sham or CPB groups at baseline.

Figure 3. ERK1/2 inhibition prevents the increased contraction of mesenteric arterioles to phenylephrine after CPB. PD98059 inhibition of the ERK1/2 pathway attenuated the increased contraction to phenylephrine post-CPB ($-62\% \pm 9.1\%$, $*P < .01$). The contraction to phenylephrine at 180 minutes was decreased compared with baseline in the sham group and was not further reduced by ERK1/2 blockade. Inhibition of p38 activity with SB203580 had no effect on contraction to phenylephrine in either the sham or CPB groups.

were monitored. The pericardial sac was opened through a median sternotomy. Pigs were given intravenous heparin (300 units/kg) and cannulated via the distal ascending aorta and the right atrium. CPB was initiated with a kaolin activated clotting time of more than 480 seconds, which was maintained. The proximal aorta was crossclamped, and cold crystalloid cardioplegic solution was infused into the aortic root. An initial 300 mL of cold high-potassium cardioplegic solution (0°C - 4°C , 25 mmol/L K^{+}) was administered followed by 150 mL of cold low-potassium cardioplegic solution (0°C - 4°C , 12 mmol/L K^{+}) each 15 minutes.

Tissue Collection

Samples of mesenteric tissue were harvested in both groups at baseline and at 180 minutes of post-CPB reperfusion. The mesenteric tissue was snap frozen in liquid nitrogen and stored at -80°C for molecular analysis or fixed in 4% formaldehyde for immunohistochemical analysis.

In Vitro Assessment of Mesenteric Microvascular Responses

Mesenteric arterioles (80-150 μm in diameter and 1-2 mm in length) were dissected from the surrounding tissue with a $\times 40$

Figure 4. Contraction to vasopressin was not changed in mesenteric microvessels after CPB. The mesenteric vasomotor responses to vasopressin were similar to the baseline after 180 minutes in both the sham and CPB groups.

microscope and examined in isolated organ chambers as previously described.⁵ Contractile responses were examined to phenylephrine (1-100 $\mu\text{mol/L}$) and vasopressin (1 nmol/L- $\mu\text{mol/L}$) in both the presence and absence of the p38 kinase inhibitor SB203580 (1 $\mu\text{mol/L}$) or the ERK 1/2 inhibitor PD98059 (30 $\mu\text{mol/L}$). Responses were recorded as percent contraction of the baseline diameter.

Western Blot Analysis

Total lysate from mesenteric tissue was obtained, separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis, and transferred to membranes as previously described.⁵ Membranes were incubated with anti-phospho-p44/p42 (ERK1/2) MAPK (New England Biolabs, Beverly, Mass) or anti-phospho-p38 (Santa Cruz Biotechnology, Santa Cruz, Calif) antibody for 2 hours, followed by the appropriate secondary antibody conjugated to horseradish peroxidase.

Immunohistochemistry

Mesenteric tissues were fixed, embedded in paraffin, sectioned (5 μm), and mounted on glass slides. Immunostaining was performed as previously described.⁵ The sections were incubated with either the polyclonal rabbit anti-phospho-ERK1/2 (New England Biolabs) or anti-phospho-p38 antibody (Santa Cruz Biotechnology), followed by biotin-conjugated antirabbit immunoglobulin G secondary antibody and avidin-biotin-peroxidase complex (Santa Cruz Biotechnology).

Statistical Analysis

Values are shown as mean $\pm$ SEM. Statistical analyses were performed by 2-factor analysis of variance for repeated measures or *t* test as appropriate.

Results

No Differences in Hemodynamics, Arrhythmia, Temperature, or Arterial Blood Gas

No significant differences were observed in heart rate or mean arterial pressure between the sham and CPB groups.

Figure 5. Contractile responses of mesenteric arterioles to vasopressin are in part dependent on the p38 pathway at baseline. Inhibition of p38 pathway activity with SB203580 decreased contraction to vasopressin at baseline in both sham and CPB groups ($-27\% \pm 4.3\%$ and $-25\% \pm 2.5\%$, respectively; $*P < .01$). ERK1/2 blockade with PD98059 showed no change in contraction to vasopressin in either sham or CPB groups at baseline.

Figure 6. Mesenteric microvessel contraction to vasopressin is in part dependent on p38 activity after CPB. After 180 minutes in the sham and CPB groups, p38 inhibition with SB203580 reduced contraction to vasopressin ($-29\% \pm 3.6\%$ and $-41\% \pm 3.5\%$, respectively; $*P < .01$). PD98059 inhibition of the ERK1/2 pathway produced no change in contraction to vasopressin in either sham or CPB groups after 180 minutes.

Incidence of arrhythmia, core temperature, and arterial blood gas measurements were not significantly different between groups (data not shown).

Increased Mesenteric Microvascular Contraction to Phenylephrine After CPB, Which Is Prevented by ERK1/2 Inhibition

Mesenteric arterioles showed increased contractile responses to phenylephrine after CPB compared with baseline ($+49.7\% \pm 5.5\%$; $P < .01$). In the sham group, contraction to phenylephrine was reduced after the sham procedure compared with baseline ($-37.0\% \pm 5.1\%$; $P < .01$; Figure 1). Inhibition of the ERK1/2 pathway with PD98059 reduced the contraction to phenylephrine at baseline in both the sham and CPB groups ($-31\% \pm 4.1\%$ and $-52\% \pm 9.1\%$, respectively; both $P < .01$; Figure 2) and prevented the increased contractile responses to phenylephrine after CPB ($-62\% \pm 9.1\%$; $P < .01$; Figure 3). The already decreased contraction to phenylephrine at 180 minutes in the sham group was not further reduced by ERK1/2 pathway blockade. p38 Inhibition with SB203580 had no effect on contractile responses

to phenylephrine in either the sham or CPB group (Figures 2 and 3).

Contractile Responses to Vasopressin Are Unchanged After CPB and Reduced by p38 Inhibition

Contractile responses to vasopressin were unchanged from baseline after 180 minutes in both the sham and CPB groups (Figure 4). p38 Pathway blockade with SB203580 reduced contractile responses to vasopressin at baseline in both sham and CPB groups ($-27\% \pm 4.3\%$ and $-25\% \pm 2.5\%$, respectively; both $P < .01$; Figure 5), as well as after 180 minutes in the sham and CPB groups ($-29\% \pm 3.6\%$ and $-41\% \pm 3.5\%$, respectively; both $P < .01$; Figure 6). ERK1/2 inhibition resulted in no change in contraction to vasopressin in either the sham or CPB groups at baseline and after 180 minutes (Figures 5 and 6).

CPB Increases ERK1/2 Activation, Whereas p38 Activity Is Unchanged

Activated ERK1/2 levels were increased by more than 40% after CPB compared with baseline. In the sham group,

Figure 7. ERK1/2 activity is increased after CPB, whereas levels of activated p38 are unchanged. Representative Western blot images of activated ERK1/2 and p38. Levels of activated ERK1/2 were increased by more than 40% after CPB compared with baseline (BL). In the sham group, there was a trend toward decreased ERK1/2 activity after the sham procedure compared with baseline (BL). Levels of activated p38 were unchanged in both the CPB and sham groups.

ERK1/2 levels were slightly decreased after the sham procedure, although the difference was not statistically significant. Levels of p38 were unchanged in both the sham and CPB groups (Figures 7 and 8).

Increased Activated ERK1/2 Localizes to Mesenteric Arterioles

In histologic sections of mesenteric tissue, activated ERK1/2 localized to arterioles and was increased after CPB compared with controls (Figure 9). Staining of activated p38 in mesenteric arterioles was unchanged after CPB compared with baseline (data not shown).

Discussion

In our study of the mesenteric microvasculature in a pig model of CPB, the main findings are as follows: (1) increased contraction of mesenteric arterioles to phenylephrine after CPB and no difference in the response to vasopressin; (2) decreased contractile response of mesenteric arterioles to phenylephrine by inhibition of the ERK1/2 pathway; (3) reduction in mesenteric microvessel contraction to vasopressin by p38 kinase blockade; and (4) elevation in activated ERK1/2 after CPB that localizes to mesenteric arterioles and no change in levels of activated p38 kinase. On the basis of these results, we conclude that mesenteric microvascular contraction to phenylephrine is in part dependent on ERK1/2 and increased after CPB through a mechanism that involves activation of the ERK1/2 path-

Figure 8. ERK1/2 activity is increased after CPB, whereas levels of activated p38 are unchanged. Histogram showing quantification of Western blot analysis of activated ERK1/2 and p38 levels in the sham and CPB groups (* $P < .01$).

way. The mesenteric contractile response to vasopressin involves p38 kinase and is unchanged after CPB.

Mesenteric ischemia is an uncommon but highly morbid complication of cardiac surgery with CPB and has been associated with patient risk factors including 3-vessel coronary artery disease, peripheral vascular disease, renal failure, and diabetes mellitus. Operative risk factors for development of mesenteric ischemia include duration of CPB and aortic crossclamp time, use of an intra-aortic balloon pump, and the use of inotropic and vasopressor support.^{1,6} The use of vasopressor agents during and after CPB appears to have variable effects on different organ systems. In a study of regional perfusion in a pig model of CPB, phenylephrine administered to treat hypotension during CPB raised the

Mesenteric ERK1/2 Activity

Figure 9. Activated ERK1/2 localizes to mesenteric arterioles, and is increased after CPB. Representative histologic sections showing mesenteric arterioles at baseline (*left*), post-CPB control stained with secondary antibody (2° Ab only; *center*), and after CPB (*right*). Staining of activated ERK1/2 is demonstrated in the mesenteric arterioles and is increased after CPB compared with baseline and the post-CPB control.

systemic blood pressure with increased vascular resistances in the mesenteric and renal vascular beds, while cerebral and femoral vascular resistances remained unchanged. This increase in mesenteric vascular resistance resulted in decreased perfusion, which was shown by reduced radioactive microsphere blood flow measurements.⁷ In studies from our laboratory, we found similar differences in vasomotor responses to CPB. In peripheral arterioles harvested from skeletal muscle of cardiac surgery patients before and after CPB, we⁵ found that the contractile responses to phenylephrine and vasopressin were reduced after CPB. In contrast, using a pig model of CPB with cardioplegic arrest, we³ observed that the contraction of mesenteric arterioles to phenylephrine was greater after CPB than before CPB. Similarly, an increased contractile response of mesenteric arteries to phenylephrine after CPB was shown in an animal model of partial CPB.⁸ Thus, the differential contractile responses to vasopressor agents used to treat hypotension after CPB likely contribute to mesenteric hypoperfusion and ischemia, inasmuch as there is reduced contraction in certain vascular territories as in skeletal muscle while the mesenteric system demonstrates greater vasoconstriction.

CPB has been suggested to cause mesenteric hypoperfusion associated with local changes in vasoconstrictor and vasodilator neurohumoral regulation. Gut mucosal hypoperfusion has been demonstrated in both animal models and patients undergoing CPB.^{9,10} Recently, poly(adenosine 5'-diphosphate-ribose) and nitric oxide deficiency have been shown to be involved in mesenteric vascular dysregulation after CPB.^{11,12} MAPK pathways also have been suggested to mediate vasomotor responses in mesenteric resistance vessels. The ERK1/2 pathway has been demonstrated to mediate the contractile response of mesenteric arterioles to angiotensin II,^{13,14} which has been proposed as a contributor to decreased mesenteric perfusion during and after CPB.¹⁵ In addition, p38 and ERK1/2 have been implicated

in the molecular pathway of contractile response of mesenteric resistance vessels to norepinephrine.^{16,17} p38 has been shown to mediate thromboxane-induced contraction of mesenteric resistance arteries.¹⁸ In a study of peripheral vasomotor function in patients after cardiac surgery with CPB, we⁵ demonstrated that MAPK pathways in part mediate a decrease in contractile responses to phenylephrine and vasopressin that were associated with reductions in ERK1/2 and p38 activity. The results of the present study are consistent with these previous findings in that changes in MAPK activity contribute to vasomotor dysfunction after CPB. However, in this study, although ERK1/2 and p38 were shown to mediate contractile responses to phenylephrine and vasopressin, respectively, ERK1/2 activity was elevated and associated with increased contraction to phenylephrine after CPB, whereas p38 activation was unchanged and we observed no increase in contraction to vasopressin after CPB. The decrease in contraction to phenylephrine in the sham group at 180 minutes is worth mention. In accordance with this finding, we observed a trend of decreased ERK1/2 activity in the sham group at 180 minutes. The trend of diminished ERK1/2 activity and reduced mesenteric contraction to phenylephrine likely involved an effect of the isoflurane gas general anesthesia. Isoflurane and sevoflurane have been associated with inhibition of ERK1/2-mediated vascular smooth muscle contraction.^{19,20}

The limitations of our study include the operative technique, the pharmacologic conditions, and the timing of vessel harvest. First, our CPB protocol was under normothermia, whereas many clinical cardiac surgical procedures involve CPB with some degree of hypothermia. This certainly may have had an impact on the vascular reactivity of the mesenteric circulation. In addition, in hypothermic CPB the process of cooling and rewarming per se may alter vascular regulation owing to the release of circulating catecholamines and other neurohumoral substances that can

affect adrenergic receptor function and the regulation of other signaling pathways. This process could conceivably further exacerbate the potential for mesenteric ischemia. Also, we wanted to examine the mesenteric vascular effects in a clinically relevant model, so we used a model of CPB with cardioplegic arrest. Although unlikely, this may have influenced the results compared with what would have been observed with CPB alone. Next, receptor signaling may be affected not only by endogenous catecholamines and other neurohumoral substances released during CPB, but also by exogenous vasodilator, vasopressor, and inotropic agents commonly used in managing patients having cardiac surgery. Thus, the true "clinical" pharmacologic milieu may be somewhat different from the "pure" experimental conditions tested in the study. Finally, the time to harvest mesenteric vessels in our model was restricted to early in the reperfusion period at 180 minutes after CPB. In postoperative patients, mesenteric ischemia may develop later in the postoperative period, at which time the neurohumoral and molecular pathways may have a different effect on mesenteric vascular reactivity to vasopressor agents.

In conclusion, using a porcine model of CPB to study the mesenteric vasomotor responses to vasopressors, we demonstrated that the contraction to phenylephrine was increased after CPB and in part mediated by ERK1/2, whereas the contractile response to vasopressin involves the p38 pathway and is not increased after CPB. The use of vasopressin as a vasopressor agent in the postoperative period after cardiac surgery with CPB may reduce mesenteric hypoperfusion and the risk of mesenteric ischemia.

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