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Vascular changes after cardiac surgery: role of NOS, COX, kinases, and growth factors

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1. ABSTRACT

Cardiovascular disease remains the leading cause of mortality in the industrialized world. Despite advances in pharmacotherapy and catheter based interventions, coronary artery bypass grafting remains an essential therapeutic modality. The majority of coronary artery bypass operations, as well as other cardiac surgical procedures require the use of ischemic cardioplegic arrest and cardiopulmonary bypass, both of which result in iatrogenic injury to the vasculature and microcirculation. This injury can manifest as impaired vasorelaxation or vasoconstriction, depending upon the organ system involved, resulting in impaired tissue perfusion and the development of edema. Key to this dysfunction are changes in the following: nitric oxide signaling secondary to changes in eNOS and iNOS expression and activity, cyclooxygenase function with increases in pro-inflammatory COX-2 activity, alterations in Protein Kinase C and Mitogen Activated Protein Kinase signaling, and an increase in Vascular Endothelial Growth Factor expression increasing vascular permeability and dilatation. This review discusses our current understanding of cardioplegia and cardiopulmonary bypass induced changes in the vasculature, and therapeutic interventions aimed at modulating the altered signaling pathways.

2. INTRODUCTION

Cardiovascular disease remains the leading cause of mortality in the industrialized world. Despite advances in pharmacotherapy and catheter-based interventions such as percutaneous transluminal coronary angioplasty and coronary artery stenting, coronary artery bypass grafting (CABG) remains a major therapeutic modality for patients suffering from coronary artery disease (CAD). The majority of CABG operations and other cardiac surgical procedures (valvular, aortic, congenital) utilize ischemic cardioplegic arrest (CP) with cardiopulmonary bypass (CPB) (54) to provide a relatively bloodless and motionless operative field for safe and successful conduct of the procedure. Despite continued refinements in myocardial protection strategies and cardiopulmonary bypass systems, cardiac surgery utilizing CP/CPB is associated with significant systemic vascular dysfunction which can result in impairments to perfusion of the brain, myocardium, lungs, intestinal tract, and skeletal muscle. These perfusion deficits may manifest as neurocognitive deficit, impairments in myocardial pump function secondary to coronary artery vasospasm or impaired coronary microcirculatory vasomotor function, persistent requirement for mechanical ventilatory support, systemic

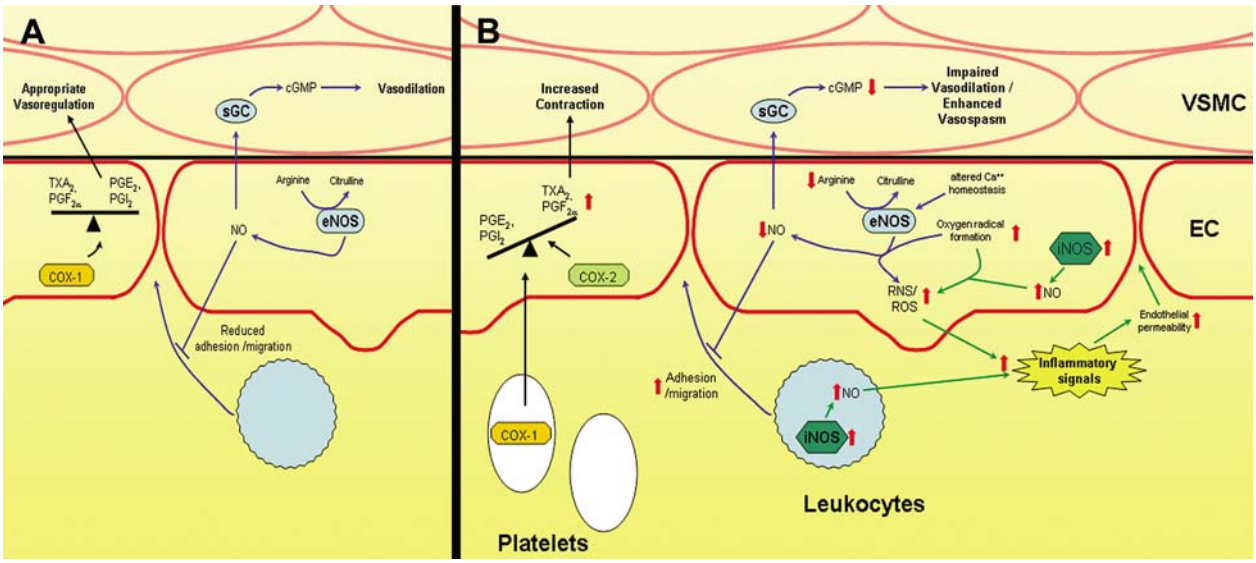

Figure 1. Alterations in vascular NOS and COX signaling pathways following cardiac surgery. A) NOS and COX signaling under normal conditions and B) following cardiac surgery Red arrows indicate relative changes following cardiac surgery. Blue arrows depict eNOS associated signaling alterations, green arrows represent iNOS dependent signaling alterations. Abbreviations used: EC – endothelial Cells; VSMC – vascular smooth muscle cells; COX – cyclooxygenase; eNOS – endothelial nitric oxide synthase; iNOS – inducible nitric oxide synthase; RNS/ROS – reactive oxygen/nitrogen species, NO – nitric oxide – sGC – soluble Guanylate Cyclase; cGMP – cyclic Guanosine Monophosphate TXA_2 – thromboxane A_2; $PGF_{2\text{-alpha}}$– prostaglandin $F_{2\text{-alpha}}$; PGI2 – prostacyclin; PGE_2 – prostaglandin E_2.

hypotension, and generalized edema secondary to increased vascular permeability.

The etiology of vascular dysfunction after CP/CPB is multi-factorial, involving a complex interaction between inflammatory mediators generated during CPB, endothelial impairments, and alterations in vascular smooth muscle contractility. Central to these changes in the vasculature after CP/CPB lie signaling alterations in nitric oxide synthase (NOS), cyclooxygenase (COX), kinase pathways, and growth factors.

3. NITRIC OXIDE SYNTHASE

Key to the vascular pathophysiology observed after CP/CPB are altered concentrations of nitric oxide (NO) (Figure 1). Nitric oxide is produced in healthy endothelial cells via activation of a constitutive nitric oxide synthase (eNOS), and in a variety of other cell types including activated endothelial cells, inflammatory cells and macrophages, cardiomyocytes, intestinal cells, and vascular smooth muscle cells by the inducible form of nitric oxide synthase, iNOS. Physiologic roles of eNOS, which is responsible for the endothelial production of NO via conversion of L-arginine to L-citrulline, include endothelial-dependent vasorelaxation through activation of guanylate cyclase, inhibition of leukocyte adhesion, and attenuation of platelet activation. In addition to these endothelial effects, eNOS also regulates tone in the vascular smooth muscle to which the endothelium signals, and thus affects medial vasodilatory responses (8).

It is well documented hyperkalemic cardioplegic arrest and ischemia-reperfusion alter endothelial structure and indices of endothelial function, most notably endothelial dependent vasorelaxation (14, 43, 62, 63). The etiology of this impairment in endothelial dependent relaxation is multi-factorial, with experimental studies demonstrating the release of NO from eNOS is reduced after ischemic cardioplegic arrest (23). When assessing eNOS activity via examination of endothelial dependent responses, the defect in function is likely due to changes in cell membrane potential (23, 31), substrate cofactor depletion (5, 12), alterations in the concentration or compartmentalization of intracellular calcium (16, 48), and injury to cell membranes, associated regulatory enzymes, or ion pumps (61). The internal thoracic artery, a common conduit for bypass grafting, actually demonstrates increases in eNOS expression after CPB, which may in part explain the relatively low incidence of vasospasm in this vessel (75). While impaired signal transduction and reduced agonist stimulated production of NO likely contribute to the reduced endothelial-dependent relaxation after cardioplegic arrest, increased degradation or binding of NO through interactions with free radicals may decrease the bioavailability of NO to the vascular smooth muscle (8). Following reperfusion after cardioplegic arrest, increased breakdown of NO occurs from increased oxidative stress secondary to the generation of oxygen-derived free radicals (40), and production is further impaired by exposure of the endothelium to fragments of activated complement (69) (24), activated neutrophils, and macrophages (60). Taken together, the diminshed production, combined with the increase in degradation / free-radical coupling, results in a

significant deficit of eNOS generated NO required for homestatic vascular function.

In contradistinction to eNOS, the inducible form of NOS, iNOS, is found in increasing quantities in the myocardium after cardioplegic arrest (20, 73), and to a lesser extent in other organs after CPB including the pulmonary vasculature (28, 57), the mesenteric vasculature (74), and brain (46). In contrast to the low concentrations of NO produced by eNOS which inhibit adhesion molecule expression, cytokine synthesis, and leukocyte adhesion, the large amounts of NO generated by iNOS under stressed local conditions can be toxic and pro-inflammatory (26). This is due to the excess spontaneous reaction of NO with the reactive oxygen radicals released under inflammatory or post-ischemic conditions by stressed endothelial cells and activated leukocytes resulting in formation of peroxynitrite, which may cause cell apoptosis, cell necrosis, and circulatory shock (7). In addition, to the proinflammatory stimulus presented by CPB and enhanced peroxynitrite formation by activated leukocytes, increased plasma nitrotyrosine in blood collected from the coronary sinus indicates enhanced peroxynitrite formation in myocardium following cardioplegia/reperfusion likely due to the associated ischemic insults (30).

There are a number of potential mechanisms of cardiovascular dysfunction induced by excess levels of peroxytnitrite (Figure 1). First peroxynitrite can directly modify numereous proteins through direct nitration/oxidation reactions (reviewed in (52, 70)). Modified proteins of particular interest in cardiovascular perturbations associated with CP/CPB include peroxynitrite-induced loss of function modifications of specific vascular regulators such as eNOS and prostacyclin synthase (58), as well as cystoskeletal and contractile elements in myocytes and/or smooth muscle cells (52). Other general effect of peroxynitrite include induction of apoptosis via upstream activation of apoptotic cell signaling cascades (MAPK's) resulting in caspase activation, and/or opening of the mitochondrial permeability transition pore and associated subsequent apoptotic effects (11, 15, 52). Peroxynitrite also causes irreversible inhibition of many proteins in the mitochondrial respiratory chain resulting in reduced ATP formation and metabolic alterations (55). Another major mechanism of peroxynitrite-induced cell damage is the production of single-strand DNA breaks which results in activation of the DNA repair enzyme poly-ADP ribose polymerase (PARP) subsequently depleting ATP levels and initiation of necrotic cell death (71).

iNOS expression can also be elevated by increased circulating levels of tumor necrosis factor-alpha (TNF-alpha), interleukin (IL)-6, IL-8, and other cytokines liberated during CPB (20) (22). In the pulmonary circulation, eNOS expression decreases significantly, but no significant changes occur in iNOS expression (57). The resulting deficit, combined with increased cyclooxygenase expression (as discussed in Section 4), can lead to significant impairments in pulmonary vasodilatation, manifesting as increased pulmonary vascular resistance. Interestingly, expression of eNOS mRNA is increased in the kidney and cerebral cortex of rats subjected to CPB (47), but the functional significance of this remains unclear.

4. CYCLOOXYGENASE

Cyclooxygenase (COX) acts in a two-step conversion process of arachidonic acid (AA) (51) to form prostaglandins (PG). Initially, COX converts AA to a cyclic endoperoxide (PGG_2) by the action of COX-1 or COX-2. This is subsequently followed by cleavage (via a peroxidase) to yield endoperoxide (PGH_2). These intermediate products of AA metabolism by COX are unstable and rapidly converted to prostaglandins (PGE_2, PGF_2, thromboxane A_2, PGI_2) by specific isomerase enzymes.

The expression of COX-2 (common in the endothelium of patients with coronary artery disease) is enhanced in the reperfusion phase following blood cardioplegia during coronary artery bypass graft surgery, while COX-1 expression remains unchanged (50, 76). Consequently, prostaglandin release (likely thromboxane A_2) is stimulated which then activates the contractile response of coronary arterioles to serotonin (Figure 1). The upregulation of COX-2 by CP/CPB also results in the generation of predominantly vasoconstrictive prostaglandins resulting in atrial and ventricular microvascular constriction (49, 50, 62). This enhanced response is due to an increased production and release of contractile prostanoids since the response is inhibited in the presence of either indomethacin or NS398, a selective inhibitor of COX-2, as well as the thromboxane A_2 synthase inhibitor U63557A (49, 50). This may lead to coronary microvessel spasm, which potentially, can contribute to myocardial ischemia and injury after surgery (Figure 1). Additionally, these prostoglandins can regulate vascular permeability in a manner similar to that of NO in part through activation of tyrosine kinase receptors and mitogen activated protein (MAP) kinases (53). The inducing factors leading to increased expression of COX-2 are most likely myocardial hypoxia and ischemia which occur during cardioplegic arrest as well as the exposure of the myocardium and blood vessels to inflammatory cytokines. In contrast to iNOS, which is not regulated by agonist stimulation or by intracellular calcium concentration, there is evidence that COX-2 is regulated by agonists such as serotonin (49, 50). Separating the effects of NO and PG when discussing changes in vasomotor activity and permeability during cardiac surgery remains difficult as they are often synergistic and complementary in their actions. In addition to coronary changes in COX-2 expression, experimental models utilizing rats undergoing CPB have demonstrated COX-2 expression is significantly up-regulated in the cerebral cortex after CPB, a finding which may have clinical implications relating to neurocognitive function after cardiac surgery (33). Increased release of constrictor prostanoids have also been implicated in the alterations in pulmonary microvascular responses after CPB (66). The mechanism underlying these findings may relate to increases in COX-2 mRNA and protein expression (no changes in COX-1 expression

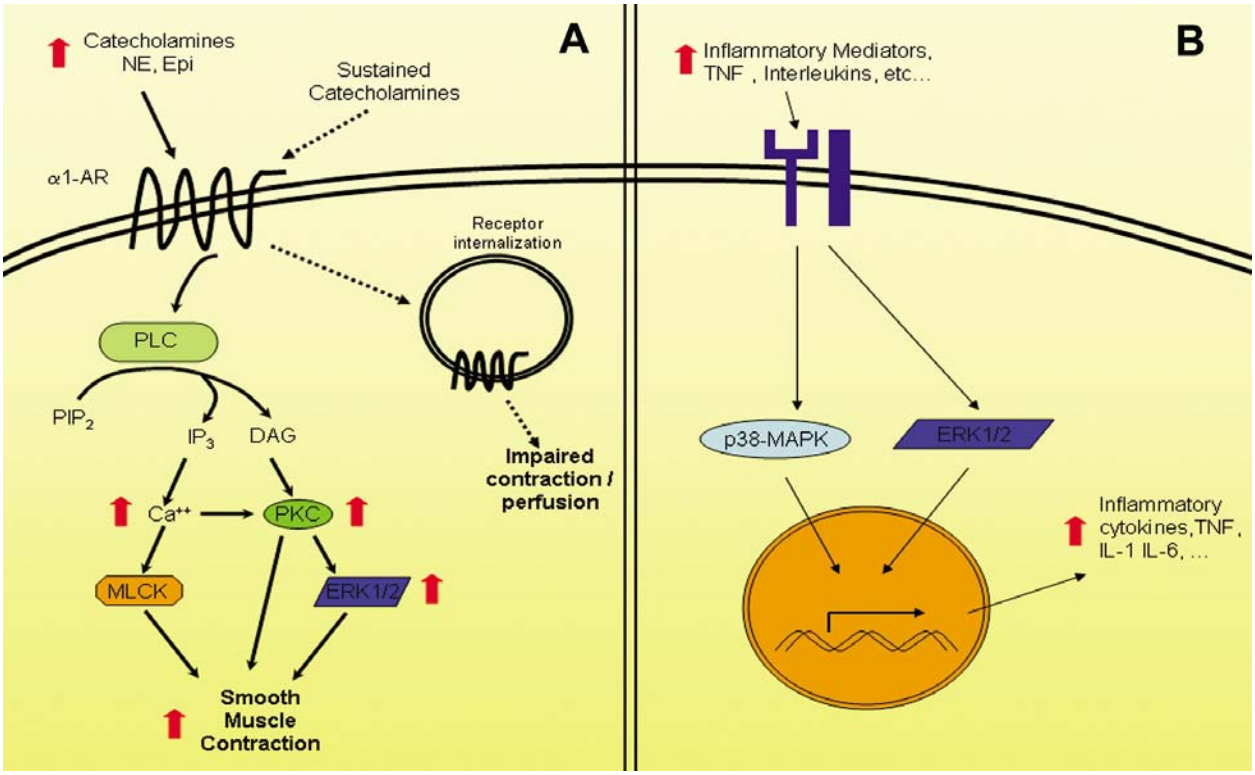

Figure 2. Alterations in (A) catecholamine signaling and (B) inflammatory mediators following cardiac surgery. A) Red arrows indicate relative changes shown to be increased following cardiac surgery. Dotted arrows represent effects associated with sustained catecholamine exposure. Early during reperfusion increased catecholamines promote smooth muscle contraction through increased activation of components of adrenergic signaling. Sustained catecholamine exposure results in down regulation of receptor signaling and impaired perfusion. B) Numerous inflammatory mediators from white cells subjected to CPB can produce increased production of inflammatory mediators from the target vascular tissues. Abbreviations: NE – norepinephrine; Epi – Epinephrine; a1-AR – alpha 1 adrenergic receptor; PLC – phospholipase C; PIP2 – phosphatidyl inositol 4,5 bisphosphate; IP3 – inositol tri-phosphate; DAG – Diacylglycerol; PKC – protein kinase C; MLCK – myosin light chain kinase; ERK – extracellular signal regulated kinase; p38-MAPK – p38 mitogen activated protein kinase, TNF – tumor necrosis factor; IL – interleukin.

were found), which have been found in the lungs and pulmonary vasculature after CP/CPB, with concominant increases in pulmonary microvascular vasoconstriction in response to serotonin. These increases in vasoconstrictive responses were partially inhibited by the COX-2 inhibitor NS398, but not the thromboxane synthase inhibitor U63557A (57). These findings implicate increases in COX-2 expression as a possible mechanism for the increased pulmonary vascular resistance often observed after CPB.

5. KINASE PATHWAYS

Much of the vascular dysfunction observed in the coronary and systemic circulation after CP/CPB relates to impairments in vascular tone and responsiveness to adrenergic agonists (Figure 2A). These impairments, which can range from diminished to excess vasoconstriction culminate in insufficient tissue perfusion (20, 80, 82). Key to this derangement are Protein Kinase C (PKC), and the downstream Mitogen Activated Protein Kinases (MAPK). The PKC family consists of 12 serine-threonine kinases, of which PKC Alpha has been identified as the predominant isoform present in the human coronary and skeletal microcirculation (67). Stimulation of the alpha-1 adrenoreceptor (G-protein receptor) results in activation of Phospholipase C resulting in conversion of phosphoinositolphosphate-2 (PIP_2), liberating 1,4,5-triphosphate (IP_3), diacylgylcerol (DAG) and calcium (59). DAG and calcium serve to activate PKC, which can augment smooth muscle contraction directly through increasing intracellular calcium (45), decreasing activity of myosin light chain phosphatase (68), or increasing myofilament sensitization to calcium, independent of increases in cytosolic calcium concentration (32). Activation of PKC also leads to activation of the MAPK ERK 1/2 (27, 78). MAPK are serine-threonine kinases involved in vasomotor function and vascular permeability and can be found in endothelial cells and vascular smooth muscle (39). Multiple stimuli have been shown to activate MAPK, including ischemia, shear stress, and vasoactive agents. The three major MAPK families implicated in cardiovascular signaling thus far include: the extracellular signal-regulated kinases (ERK), the c-Jun-NH_2-terminal

Table 1. Location specific changes in vasomotor regulation following cardiac surgery

Circulatory Bed	Vascular Response	Potential Mechanism
Coronary	Impaired vasomotor regulation. Increased or decreased vascular tone Vasospasm	Altered NO and COX signaling, increased VEGF Insults associated with cardioplegic arrest
Pulmonary	Enhanced SM and EC contraction Increased permeability and vascular tone	Inflammatory signals associated with CPB ERK and PKC activity Altered eNOS activity
Peripheral (Skeletal)	Hypotension/ impaired vasomotor regulation	Altered NO and COX signaling Sustained catecholamines
Gastrointestinal (mesenteric)	Enhanced constriction	Inflammatory signals associated with CPB ERK and PKC activity Altered NO and COX signaling

protein kinases (JNK), and p38 kinase. Of the three, ERK 1/2 is thought to play the most significant role in postoperative vascular dysfunction, given it can regulate endothelial cell permeability / edema formation (10, 35), myogenic tone (39) and contractile responses of microvessels to phenylephrine and vasopressin (36).

Postoperatively, the endogenous adrenergic stress response to CPB results in a release of vasoactive catecholamines, which act on alpha-$_1$-adrenoreceptors, including norepinephrine and epinephrine (34, 79). A sustained increase in circulating levels of catecholamines *in vivo*, or prolonged exposure to catecholamines *in vitro* results in subsequent loss of alpha-$_1$-adrenoreceptor mediated vascular smooth muscle cell contraction, and diminished IP_3 turnover, which results in decreased concentrations DAG and calcium, both of which are required for conventional PKC activation (41, 56). The resulting lack of DAG and calcium may account for the decreases in PKC activity seen in coronary and skeletal microvessels after CP/CPB (67). Decreases in PKC activity, which functions in activating MAPK, specifically ERK 1/2 (27, 78), can lead to decreases in ERK 1/2 activity as has been demonstrated after CP/CPB in the coronary and skeletal microcirculation (36, 39), culminating in impaired microvascular myogenic tone and vasoconstriction.

Compounding the vascular dysfunction induced by the effects of CP/CPB on kinase activity, are signal transduction mechanisms triggered by a broad range of effectors including TNF-alpha, which is itself elevated after CP/CPB (44), and can lead to ERK 1/2 and MAPK activation in turn inducing IL-6 production (29), leading to a cascade of inflammatory events that include leukocytosis, thrombosis, and lymphocyte activation (77). The predominance of TNF-alpha's stimulatory effect on MAPK remains unknown as the overall affect of CP/CPB on ERK 1/2 seems to be stimulatory (Figure 2B). In the pulmonary circulation, rapid induction of ERK 1/2 (37) leads to enhanced vasoconstriction likely contributing to the elevations in pulmonary vascular resistance commonly seen after CP/CPB. In the mesenteric microcirculation, key to the pathophysiology of mesenteric ischemia, ERK 1/2 activity and expression levels are markedly increased after CPB with significant augmentation of responses to phenylephrine (38).

6. GROWTH FACTORS

The predominant growth factor involved in vascular changes after CP/CPB has been identified as Vascular Endothelial Growth Factor (VEGF), a potent vasodilator and inducer of vascular permeability operating through the tyrosine kinase regulated release of NO (64, 72). The expression of VEGF and that of it's receptor VEGFR-1, are significantly up-regulated after CP and reperfusion, resulting in increased coronary microvascular relaxation responses (6, 64, 72, 75). Conversely, coronary microvascular relaxation responses to adenosine diphosphate (ADP), an endothelial-dependent vasodilator, are unchanged after CP, suggesting the up-regulation of VEGF receptors on the coronary endothelium is selective and may play a role in mediating perioperative increases in vascular permeability. Porcine studies involving CP/CPB have demonstrated increases in VEGF in the lungs after CPB, possible contributing to the post-operative lung edema observed in patients after CPB (65). The increases in circulating levels of VEGF after CP/CPB have been correlated with clinical cardiovascular impairments, as well as the development of capillary leak syndrome (1, 21).

Basic fibroblast growth factor (bFGF), another growth factor capable of inducing micrvascular relaxation, increases in concentration in the plasma after CP/CPB (2), but remains unchanged in the myocardium and skeletal muscle (72). The coronary and skeletal microvascular relaxation responses to bFGF also remain unaltered after CP/CPB, indicating it may play little, if any role in postoperative vasomotor dysfunction after CP/CPB (72).

7. PERSPECTIVE

The clinical manifestations of CP/CPB induced vascular dysfunction are wide ranging (Table 1). We commonly observe decreased basal tone and decreased alpha-adrenergic microvascular responses in the peripheral/skeletal muscle vascular beds which manifests as systemic hypotension, often necessitating the use of adrenergic vasopressors to maintain adequate tissue perfusion pressure (36, 72, 73). In the coronary circulation, impairments in smooth muscle contraction lead to decreased coronary vascular tone in certain patients (67, 81), whereas decreased endothelial-mediated relaxation can lead to an increased propensity to spasm in others (49, 50). The pulmonary circulation demonstrates increased microvascular contractile responses, which in addition to edema from increased endothelial permeability, result in increased pulmonary vascular resistance and shunting (24, 57). Similar increases in vasoconstrictor responses arise in the mesenteric microcirculation (38) predisposing to the

development of mesenteric ischemia, particularly when vasoactive drugs are administered to regulate blood pressure after CPB (3).

Clinical interventions to limit the degree of vascular dysfunction have attempted to manipulate the previously described alterations after CP/CPB. The increased expression of COX-2 and enhanced contractile response of coronary arterioles to serotonin after CPB and cardioplegia (49, 50) indicate the improvements in coronary bypass graft patency obtained from the perioperative administration of aspirin may not only derive from its effects on prevention of platelet aggregation and thrombus formation (17, 25, 42), but also from the prevention of coronary spasm and preservation of microvascular flow as a result of COX-2 inhibition. The advantages of blood over crystalloid cardioplegia in emergency or high-risk cases may result from the inhibitory effects of blood on oxygen-derived free radical generation, improved coronary endothelial oxygenation, enhanced buffering capacity from histidine and other blood proteins, and better preservation of the morphology of coronary endothelial cells (60, 62). The administration of glucocorticosteroids during CPB was thought to theoretically block the effects of inflammatory cytokines and the expression of iNOS and COX-2, but has not resulted in significant clinical benefit (4, 9). Attempts to improve the bioavailability of NO in the coronary circulation have led to multiple studies investigating the supplementation of L-arginine to cardioplegic solutions. These trials have demonstrated benefits to L-arginine supplementation, including reduced release of biochemical markers of myocardial damage (13), reduced IL-2 receptor, IL-6, and TNF-alpha expression (18), and better hemodynamic performance with shorter intensive care unit stays (19). Additional investigation is needed to further examine the impact of these and other mechanistically based interventions on the clinical outcomes after cardiac surgery utilizing cardioplegic arrest and cardiopulmonary bypass.

8. CONCLUSION

Cardioplegic arrest and cardiopulmonary bypass are associated with marked systemic changes in vascular function which can lead to significant derangements in end-organ perfusion. Central to these alterations are changes in expression of nitric oxide synthases, increases in COX-2 signaling, alterations in PKC and MAPK signaling, and increased VEGF induced vasorelaxation and endothelial permeability. Therapeutic interventions aimed at these targets have met with limited proven success thus far, with the exception of aspirin therapy which may prevent of coronary spasm and preserve microvascular flow as a result of COX-2 inhibition. Despite the dramatic degree of change in vascular physiology observed after cardiac surgery utilizing cardioplegic arrest and cardiopulmonary bypass, the vast majority of patients are able to undergo surgery safely without significant complication.

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Abbreviations: AA: Arachidonic Acid, bFGF: basic Fibroblast Growth Factor, CABG: Coronary Artery Bypass Grafting, CAD: Coronary Artery Disease, cGM: cyclic Guanosine Monophosphate, CP: Cardioplegia, CPB: Cardiopulmonary Bypass, COX: cyclooxygenase , DAG: Diacylglycerol, ERK: Extracellular Signal Related Kinase, JNK: c-Jun: Nh2-terminal protein kinase, Il: Interleukin, IP3: inositol 1,4,5-tri phosphate, eNOS: endothelial Nitric Oxide Synthase, iNOS: inducible Nitric Oxide Synthase, MAPK: mitogen activated protein kinase, MLCK: Myosin Light Chain Kinase, NO: Nitric Oxide, PG: prostaglandin, PIP2: phosphoinositolphosphate-2, PKC: Protein Kinase C, PKG: cGMP Dependent Protein Kinase, RNS: Reactive Nitrogen Species, ROS: Reactive Oxygen Species, TNF: Tumor Necrosis Factor, TXAa: Thromboxane A2, VEGF: Vascular Endothelial Growth Factor

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