

Vascular changes after cardiopulmonary bypass and ischemic cardiac arrest: roles of nitric oxide synthase and cyclooxygenase

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Abstract

Cardiac surgery involving ischemic arrest and extracorporeal circulation is often associated with alterations in vascular reactivity and permeability due to changes in the expression and activity of isoforms of nitric oxide synthase and cyclooxygenase. These inflammatory changes may manifest as systemic hypotension, coronary spasm or contraction, myocardial failure, and dysfunction of the lungs, gut, brain and other organs. In addition, endothelial dysfunction may increase the occurrence of late cardiac events such as graft thrombosis and myocardial infarction. These vascular changes may lead to increased mortality and morbidity and markedly lengthen the time of hospitalization and cost of cardiac surgery. Developing a better understanding of the vascular changes operating through nitric oxide synthase and cyclooxygenase may improve the care and help decrease the cost of cardiovascular operations.

Key words

- Nitric oxide synthase
- Cyclooxygenase
- Heart surgery
- Endothelium
- Microcirculation

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Introduction

Refinements in methods of myocardial protection and operative techniques have improved the results of cardiovascular surgery. Yet, the operative treatment of cardiac diseases remains associated with systemic inflammation and a suboptimal outcome in many patients. These inflammatory changes are manifested by systemic hypotension, myocardial failure, increased vascular permeability and consequent dysfunction of organs such as the lungs, gut and brain. This is especially true when operations are performed on patients suffering acute myocardial ischemia, on patients in cardiogenic shock with

reduced peripheral perfusion, or during prolonged extracorporeal perfusion. It has been estimated that 1 to 3% of patients suffer from coronary spasm and develop temporary ST segment elevations on EKG within hours of cardiovascular surgery (1-3). However, these patients generally suffer from atherosclerotic coronary vascular disease and have several other risk factors for endothelial dysfunction. Thus, these patients suffer from a baseline impairment of nitric oxide synthase (NOS). Equally important is the deterioration in cardiac function that frequently occurs within several hours of operation (4). Increased vascular permeability, impaired regulation of myocardial perfusion, and sys-

temic inflammation undoubtedly play a major role in this negative response to surgery (5,6).

The signs of altered vascular permeability and function are common after surgery in the coronary, pulmonary and other vascular beds. However, the mechanism has only recently been understood to be a consequence of numerous simultaneous pathological stimuli including activation of complement, activation and adherence of neutrophils, macrophages, and platelets, and increased oxidative stress. These pathologic processes are mediated in part by the increased release of nitric oxide (NO) due to expression of iNOS, and the release of other inflammatory substances such as thromboxane A₂ as a consequence of expression of inducible cyclooxygenase (COX-2). These inflammatory mediators may potentially lead to changes in vasomotor regulation, endothelial integrity and vascular permeability that may compromise the recovery of patients undergoing cardiovascular surgery (6). This review briefly details the effects of ischemic cardioplegia under conditions of extracorporeal perfusion on the function and expression of NOS and COX.

Cardioplegia and endothelial function

Effects on nitric oxide synthase

It is well documented that hyperkalemia and ischemia-reperfusion alter endothelial structure and indices of endothelial function, most notably endothelium-dependent relaxation (7-10). After hyperkalemic arrest, endothelium-dependent relaxation is moderately impaired (7), possibly due to changes in membrane potential (11,12), NOS substrate and cofactor depletion, alterations in concentration or compartmentalization of intracellular calcium (13,14), or injury to the cell membranes and associated enzymes and ion pumps. In addition, the release of NO

from the constitutive isoform of NOS (eNOS) is reduced after ischemia and cardioplegia, as determined by direct measurement (15). During the initiation of reperfusion after ischemic arrest, injury to the endothelium (and presumably eNOS) is observed and is due to increased oxidative stress caused by the generation of oxygen-derived free radicals (16), exposure of the endothelium to activated complement (17,18), or the action of activated neutrophils and macrophages (6). When assessing eNOS activity by examining endothelium-dependent responses, uncertainty remains regarding whether the defect in enzyme function is due to an actual impairment in enzyme activity or to impaired signal transduction as a consequence of receptor or membrane injury. In addition, while impaired signal transduction and reduced agonist-stimulated production of NO likely contribute to the reduced endothelium-dependent relaxation after cardioplegia, increased degradation or binding of NO through interactions with free radicals may decrease the bioavailability of NO to the vascular smooth muscle (19).

Causes of impaired endothelial function and vascular permeability

Increased oxidative stress as a result of ischemia and/or reperfusion may produce direct and indirect injury to endothelial cells. The addition of either manganese superoxide dismutase or deferoxamine to a hyperkalemic cardioplegic solution markedly reduces the impairment of endothelium-dependent relaxation (20). This is presumably due to an inhibition of the generation of superoxide anion, the hydroxyl radical, and other free radicals. Furthermore, the interaction of superoxide anion and NO causes the formation of the peroxynitrite radical that has been implicated in mediating some of the free radical-induced injury. Whereas the expression of the constitutive isoform of NOS is generally not altered in vessels by brief

(<2 h) crystalloid cardioplegia and reperfusion, expression of iNOS is increased (21). The increased release of NO due to expression of iNOS may not only contribute to the reduced activity of eNOS, but may also lead to reduced peripheral vascular resistance, increased vascular permeability (22), and other manifestations of systemic inflammation after cardiopulmonary bypass.

Abundant evidence supports a role for leukocytes in mediating much of the myocardial and systemic response to cardiovascular surgery. Leukocytes may contribute to much of the myocardial and endothelial damage after ischemia (23), hyperkalemic cardioplegia (21,24), and cardiopulmonary bypass (CPB) (25). Indeed, focal leukocyte-endothelial adherence has been observed on transmission electron microscopy following cardioplegia and reperfusion (7) and improved recovery of myocardial function and perfusion has been demonstrated when leukocyte-depleted blood has been used to reperfuse hearts after cardioplegic arrest (26). In addition, monoclonal antibodies to adhesion molecules (26) or chemotactic complement fragments (21) have beneficial effects on vascular recovery when administered prior to reperfusion. Activated leukocytes may cause endothelial dysfunction through the release of oxygen-derived free radicals, proteolytic enzymes, and inflammatory cytokines. Expression of P-selectin is initiated shortly after initiation of CPB or ischemia (27). This initiates the process of polymorphonuclear neutrophil (PMN) rolling, firm adherence and activation of PMN's. Subsequently, other adhesion molecules are up-regulated, leading to the infiltration of PMN's into the perivascular tissues. PMN and macrophage infiltration has been documented in myocardium (28) after ischemia and cardioplegia and also in mesenteric (25) and pulmonary tissue (29) after CPB. Furthermore, increased circulating and tissue levels of tumor necrosis factor (TNF)-alpha, Il-6, Il-8, and other inflammatory cytokines liber-

ated during and after CPB (30-33) have been directly associated with the increase in permeability of blood vessels (6,30,31) and contribute to the inflammatory reaction and increased expression of inducible iNOS (34).

Another cytokine implicated in regulating vascular permeability is vascular endothelial growth factor (VEGF), also known as vascular permeability factor. VEGF is an extremely potent vasodilator, operating through the tyrosine kinase-regulated release of NO (35,36). VEGF expression, as well as the expression of its flk-1 receptor, is increased after blood cardioplegia-reperfusion (35) or brief (15 min) unprotected warm ischemia (37). Interestingly, the microvascular relaxation response to exogenous VEGF is increased after blood cardioplegia, while that to another endothelium-dependent vasodilator, adenosine phosphate (ADP), is not changed (35). This suggests that a selective upregulation of functional VEGF receptors on the endothelium may contribute to increase vascular permeability and systemic inflammation.

The alternative complement pathway is activated during cardiovascular surgery, when blood interacts with components of the extracorporeal circuit (38). The classical complement cascade (39) may be activated during myocardial ischemia or cardioplegia and may affect the recovery of cardiac function during surgery. The anaphylotoxins C3a, C4a, and C5a are released, which have been implicated in increasing neutrophil chemotaxis and adherence and in mediating some of the systemic inflammation associated with CPB (25,38). In addition, most anaphylotoxins possess vasoactive properties of their own in addition to directly and indirectly affecting other mechanisms of vascular regulation. Thus, anaphylotoxins may cause vascular and myocardial injury and reduce myocardial perfusion. Some complement fragments such as C5b-9, the terminal membrane attack complex, may impair endothelial cell function by direct contact and mem-

brane injury, in addition to increasing aggregation and chemotaxis of neutrophils (17). Exposure of isolated vessels to zymosan-induced complement-activated serum significantly reduces NO-mediated endothelium-dependent relaxation (17,18,40), suggesting that activated complement directly causes endothelial injury even in microvessels isolated from other blood components and cellular elements. Complement activation may cause upregulation of adhesion molecules (17) and increased generation of oxygen-derived free radicals (41). The administration of heparin (42) to anticoagulate patients prior to cannulation and systemic cooling (43) is probably protective against the effects of complement activation. However, the administration of protamine to reverse the effects of heparin is a potent activator of complement.

Changes in activity and expression of cyclooxygenase

Not only does cardioplegia-reperfusion lead to the impaired stimulated release of NO, but it also induces the expression of the inducible isoform COX-2 that has been implicated in mediating much of the local and systemic inflammation in many disease states. Increased expression of COX-2 may cause an enhanced contractile response to serotonin of human atrial (44) or porcine ventricular (8) microvessels. 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 (44). Subsequently, expression of COX-2 was documented to be increased in response to either ischemia or cardioplegia (44) which may affect both microvascular tone and permeability in part through the activation of tyrosine kinase receptors and mitogen-activated protein (MAP) kinase (45). 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 (44). Prostaglandins (PG) are formed by the action of COX in a two-step conversion of arachidonic acid (46). First, the enzyme converts arachidonic acid to a cyclic endoperoxide (PGG₂) by the action of COX-1 or COX-2, which is then followed by a peroxidase that cleaves the peroxide to yield endoperoxide (PGH₂). These unstable intermediate products of arachidonic acid metabolism by COX are then rapidly converted to the prostaglandins (PGE₂, PGF₂, thromboxane A₂, PGI₂) by specific isomerase enzymes. The inducing factors leading to increased expression of COX-2 are most likely myocardial hypoxia and ischemia that occur during cardioplegic arrest and the exposure of myocardium and blood vessels to inflammatory cytokines. While COX-1 traditionally was thought to be expressed constitutively and COX-2 was felt to be inducible, it has recently been determined that COX-2 may be significantly expressed, or may be the predominant isoform, in the lungs of certain species, just as iNOS may be constitutively expressed (47) under basal conditions in the gut. It is difficult to separate the effects of NO and prostaglandin substances when discussing changes in the regulation of vasomotor activity and permeability during cardiovascular surgery, since they are often synergistic and complementary in their actions.

Smooth muscle control of vascular tone

The changes in the expression and activity of both NOS and COX may affect coronary and peripheral vasomotor tone during and after cardiac surgery. In addition to endothelial mechanisms, the regulation of coronary blood flow is determined by metabolic, autonomic, and myogenic mechanisms which are largely characteristics intrinsic to the vascular smooth muscle. Metabolic con-

tol is mediated by the local release of vasoactive substances and increased probability of potassium channel opening (48), whereas myogenic mechanisms are based more on the intrinsic property of vascular smooth muscle to regulate vascular resistance in response to changes in transmural pressure. Experimentally, coronary microvascular myogenic contraction is preserved during extracorporeal circulation, but the pressure-diameter relation is shifted upward, suggesting a decrease in vascular tone (49). Indeed, the upward shift in the pressure-diameter relation is normalized in the presence of N^G -nitro-L-arginine, suggesting that much of this loss of tone is due to increased basal release of NO from iNOS. Furthermore, autonomic control and myogenic contraction of peripheral arterioles are impaired after CPB (50,51). Alpha-adrenergic and protein kinase C (PKC)-mediated contraction in response to phorbol ester are impaired in skeletal muscle microvessels subjected to prolonged CPB (50). This probably accounts for much of the hypotension observed for several hours to days after extracorporeal circulation. As discussed above, the cause of this alteration in intrinsic tone is multifactorial but likely to be related to the increased circulating levels of vasodilatory substances, adrenergic receptor desensitization and uncoupling from second messenger mechanisms, and the release of cytokines during cardiopulmonary bypass (6,32) that are capable of increasing expression of iNOS (34).

While increased blood flow is generally considered to be a beneficial response, maintenance of organ perfusion could be compromised by concomitant systemic hypotension, necessitating vasopressor drugs. This may result in a poor distribution of organ perfusion. Interestingly, while alpha-adrenergic and PKC-mediated microvascular responses are reduced in some vascular beds after CPB, the response to phenylephrine (an alpha-adrenergic agonist) is actually increased in the mesenteric microcirculation

(25), increasing the probability of mesenteric ischemia when vasopressor drugs are administered after CPB in order to maintain peripheral blood pressure. Thus, changes in both vasomotor tone and vascular permeability are regulated by NOS and COX. Changes in the expression and activities of these enzymes have a major impact on the recovery of patients after cardiovascular surgery, especially those subjected to extracorporeal circulation.

Prevention of microvascular injury

The use of systemic cooling and the administration of heparin (both of which decrease complement activation), utilization of blood-containing cardioplegic solutions (8,52,53) and other modifications have a beneficial effect on endothelium-dependent relaxation, myogenic contraction and responses to adrenergic agonists, and other indices of vascular health during ischemic arrest. However, a clinical benefit of blood cardioplegia or of the addition of metabolic enhancers to a cardioplegic solution has been difficult to demonstrate under non-ischemic conditions (54,55). The mechanism of the positive effect of blood is uncertain but may be due to its potent inhibitory effects on oxygen-derived free radical generation, improved oxygenation of tissues (thereby decreasing the amount of vascular ischemia), buffering capacity of histidine and other blood proteins or preservation of morphological integrity of the endothelium (6,8). The addition of blood or magnesium to the cardioplegic solution prevents the depolarization and intracellular calcium accumulation during ischemic arrest, most likely by limiting calcium entry to the cytosol from the sarcoplasmic reticulum and extracellular space (56), and by limiting the derangement in responses mediated by the endothelium and those acting directly through the vascular smooth muscle (57). In addition, the use of continuous warm blood cardioplegia has

been shown to better preserve coronary endothelium-dependent relaxation (53), although other studies have failed to demonstrate an improved effect over standard intermittent, cold blood cardioplegia (58). A key to this disparity between experimental and clinical effects may be related to species variations, differences in experimental and clinical conditions, and a negligible importance of each individual factor in the overall outcome of patients undergoing CPB. It is intuitive that the injury to vascular function, including activity of eNOS and predominance of the COX-1 isoform, may be prevented by limiting the factors that initiate the changes, namely, hypoxia, ischemia, or exposure to cytokines and activated leukocytes. Antiproteases such as aprotinin, in addition to decreasing blood loss after cardiopulmonary bypass, may be useful in reducing postoperative systemic inflammation (59). Finally, ischemic preconditioning may be useful in limiting vascular injury, since it has been reported to maintain endothelium-dependent relaxation and β -adrenergic regulation (37,60).

Clinical implications

Reduction of endothelium-dependent relaxation and other changes in vasomotor regulation following cardioplegia may predispose to coronary constriction or spasm and alter the normal regulation of myocardial perfusion in the postoperative period. Recent work demonstrating an increased expression of COX-2 and increased contractile response of coronary arterioles to serotonin suggests that the administration of anti-platelet drugs such as aspirin improves short-term coronary bypass graft patency not only by

preventing platelet aggregation and thrombus formation, but perhaps also by improving graft blood flow by preventing reductions in myocardial perfusion and changes in vasomotor regulation.

Inhibition of factors suspected in causing systemic inflammation has been attempted with corticosteroids to block the effects of inflammatory cytokines and expression of iNOS, COX-2, and other inflammatory enzymes. PMN activation has also been inhibited in some studies, but in general the results of these efforts have been disappointing. Inhibition of neutrophil infiltration with a monoclonal antibody to C5a has resulted in improved endothelial-dependent relaxation, but without functional benefits in myocardial, pulmonary, or mesenteric recovery. However, these experiments only assessed the short-term effects of impaired neutrophil adhesion and sequestration, and long-term effects were not examined. Of considerable interest is the observation that many of the clinical manifestations observed after cardiac surgery were ascribed to CPB. Yet many of these manifestations such as systemic hypotension, cognitive dysfunction, postoperative atrial fibrillation and increased vascular permeability are observed even during "off pump" coronary artery bypass grafting, or "OP CAB", in which cardiopulmonary bypass is not utilized. Nevertheless, increasing the understanding of vascular changes during cardiac surgery and developing methods to reduce microvascular injury to the heart, brain and other organs through the use of specific tyrosine kinase and guanylate cyclase inhibitors that regulate permeability, or selective inhibitors or modulators of iNOS or COX-2 will certainly be a challenge in future investigations.

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