Targeting the Endothelium

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ABSTRACT

The endothelium is an active organ with paracrine-endocrine capabilities that directs a multitude of physiological actions both locally and remotely. Cardiac arrest and resuscitation is a model of whole body ischemia reperfusion injury, interventions that have their basis in cytoprotection, reduction of the inflammatory cascade, fibrinolysis and improvement of microvasculature blood flow target the endothelium. This presentation will review pharmacologic, cell targeted therapies and periodic acceleration (pGz) interventions that have the endothelium in part as the target organ. The clinical potential of such interventions as preconditioning, conditioning and postconditioning strategies associated with cardiac arrest will be defined.

Keywords: endothelium, periodic acceleration (pGz), cardiac arrest, cardiopulmonary resuscitation, ischemia reperfusion, nitric oxide, shear stress

Introduction

The vascular endothelium is composed on 1-6 x10¹³ cells and covers an area of 1-7 m² in humans. It is uniquely localized to be a sensor and an effecter. Its location on the luminal surface of every blood vessel and the heart allows these cells to respond to humoral factors and blood flow. The interaction of the endothelium with its neighbouring cells (vascular smooth muscle cells as well as cardiomyocytes) allows for the regulation of a host of functions. Prominent among those functions are vasomotor tone, local and systemic coagulation profiles, and inflammation. Endothelial regulation of critical functions and interactions with almost all organs of the body attest to its importance in homeostasis. (1)

The endothelial cell (EC) responds to local blood flow patterns. Laminar shear stress is the undisturbed tangential flow which runs across the upper surface of the EC and pulsatile shear stress is the stress generated perpendicular to the EC surface with each heart beat. The EC response to both laminar and pulsatile shear has been assessed in several publications. Both increased laminar shear stress and pulsatile shear stress elicit EC production of vasoactive substances such as endothelial derived nitric oxide (eNO), endothelial derived hyperpolarizing factor (EDHF), prostaglandins, adrenomedullin and tissue plasminogen activator (tPA). (2-7) Marked decrease of laminar shear stress and turbulent flow produces an EC response that increases endothelin-1 (a potent vasoconstrictor) and tips the balance of pro-coagulant / anti-coagulant to favour the former. (8-9) Nitric Oxide (NO) is generated by NO synthases (NOS) which catalyze the conversion of l-arginine to NO and l-citrulline. It is important to differentiate the three nitric oxide synthase isoforms and their functional aspects. The endothelial derived nitric oxide synthase (eNOS) is constitutively expressed, calcium calmodulin dependent, and causes release of eNO in nanomolar quantities into the circulation. The release of NO occurs intermittently in these small amounts. eNO is a potent vasodilator as well as an important intracellular signalling molecule that can regulate sarcoplasmic reticulum calcium influx and decrease mitochondrial oxygen consumption. (10-13) Additionally, eNO has anti-inflammatory properties through suppression of nuclear factor kappa beta (NF-κB) activity. The latter orchestrates the inflammatory cascade. (14,15) Inducible nitric oxide synthase (iNOS) mostly present in macrophages and neutrophils produces continuous release of iNO in micromolar quantities over protracted time periods in response to inflammatory cytokines. Large quantities of nitric oxide induce severe vasodilatation and are in part responsible for intractable hypotension during sepsis. Neuronal nitric oxide synthase (nNOS) constitutionally releases small quantities of NO and is found in central and peripheral neuronal tissue as well as cardiomyocytes. The function of nNOS remains under investigation but in the heart it regulates chronotropy and may play a protective role in the myocardial response to injury. (16-21)

Endothelium and Ischemia Reperfusion Injury

Ischemia reperfusion (I/R) injury occurs...
after partial or total cessation of blood flow to an organ followed by restoration of blood flow. The inflammatory and oxidative stress cascade elicited by these events is extensive and in large part the endothelium is a key player during and after I/R. Endothelial activation by leukocytes during reperfusion induces a pro-coagulant, pro-inflammatory and pro-oxidant status that ultimately leads to endothelial dysfunction and inability to regulate local blood flow to vital organs. The greatest effects of I/R injury to the endothelium takes place at the microvasculature level.

Cardiac Arrest and Resuscitation a Model of Whole Body Ischemia Reperfusion Injury
Cardiac arrest and resuscitation has been shown by our laboratory and others to be a model of whole body I/R injury. (22-24) Depending upon the etiology of cardiac arrest, there is a period of no flow (ventricular fibrillation or asystole) or low flow (asphyxial cardiac arrest or hemorrhage) followed by a period of partial restoration of flow (chest compression, fluid/blood administration), and if successful restoration of the intrinsic heart beat and blood flow. These events are similar to those that occur during focal ischemia in heart, brain and other organs. The duration of each phase is variable and the extent and severity of the injury related in large part to the initial two phases (span of time in cardiac arrest or low blood flow and the response to resuscitation). Analogous to focal I/R injury, there are interventions that promote an innate protective organ response which when initiated can reduce or ameliorate the injury. Interventions performed prior to focal ischemia or cardiac arrests are termed preconditioning strategies (24-25) while those initiated during the ischemia or resuscitation phase are termed conditioning or per-conditioning. (26-28) Interventions performed after restoration of organ blood flow are termed postconditioning strategies. (29-33) The latter can be performed immediately upon restoration of blood flow (early postconditioning) or minutes or hours after restoration of blood flow (delayed postconditioning). (34)

Preconditioning strategies to reduce injury from focal I/R, e.g., acute myocardial infarction, have been thoroughly investigated but rarely put into practice due to the inability to predict when such event might occur. However, for pre-planned I/R such as cardiac bypass surgery, preconditioning with anaesthetics is currently clinically utilized. Deciphering the biochemical and cellular mechanisms of protection has been important in understanding pharmacologic protective strategies. The outcomes from postconditioning strategies depend upon the intervention used. (35-41) Following the successful outcome of CPR, delayed postconditioning has the potential for clinical application. (27,28)

The Endothelium during Cardiac Arrest and Resuscitation
Evidence that the endothelium plays an important role during and after cardiac arrest comes from both animal models and clinical studies. In animal models of cardiac arrest, impaired microcirculation, endothelial leakage, (42) and elevated serum levels of TNF-α and intracellular adhesion molecule-1 (ICAM-1) occur. (43-45) Studies of human survivors and non-survivors of cardiac arrest reveal increased levels of endothelin-1 (46) pro-coagulant activities, soluble endothelial adhesion molecules, complement, leukocyte endothelial interaction, (47-48) soluble selectins, von Willebrand factor and evidence of a systemic inflammatory response. (49-50) These findings and others have led to the hypothesis that postresuscitation syndrome may be analogous to a ‘sepsis-like syndrome’. (51-53)

Methods for Stimulating and Interacting with the Endothelium
Pharmacologic Interventions
Pharmacologic interventions that induce endothelial production of eNO such as statins and type V phosphodiesterase inhibitors, (54-58) those which decrease or block endothelial leukocyte interactions (peroxisome proliferator-activated receptor beta/delta (PPAR-β), (59-61)) and thrombolysis (tissue plasminogen activator) (62-63) have been explored in myocardial I/R injury and cardiac arrest with variable results. These pharmacologic interventions for preconditioning and postconditioning have limited success in part due to the poor distribution of these compounds in pharmacological significant activity to the microvasculature, the largest and most adversely affected region during I/R. Erythropoietin (EPO) is a cytoprotective molecule which in addition to having well known hematopoietic effects also has non-erythropoietic effects. (64-66) EPO enhances eNO production by phosphorylation and activation of eNOS in the endothelium. (67) Additionally, EPO mobilizes endothelial progenitor cells, which are important in endothelial repair after I/R. (68,69) EPO has been used in animal models of both focal myocardial I/R and cardiac arrest during resuscitation as a conditioning strategy as well as in pre- and postconditioning. Animal studies have shown that cardio and neuro protection occur when EPO is administered for preconditioning strategy or administered during resuscitation from cardiac arrest as well as reduced prevalence of arrhythmias when given prior to focal I/R. (67,70-75) EPO administered during cardiac arrest and resuscitation in early human trials appears encouraging. (76-77)

Gene Interventions
Endothelium targeted gene therapies for several cardiovascular diseases are being explored in animal models. Overexpression of specific targets such as eNOS, antioxidants, inhibitors of cell adhesion molecules and NF-κB form the basis for such therapies. (78-79) Inhibition of NF-κB activity via gene decoy before coronary ligation reduces infarct size by inhibiting pro-inflammatory and cell adhesion molecule expre-
such therapies will require both preclinical and clinical confirmation, but their contribution to elucidating protective endothelial mechanisms is important.

**Mechanical Intervention**

Periodic Acceleration (pGz) is the application of repetitive sinusoidal motion of the body in a head to foot direction. It is imparted with a motion platform that passively sets the whole body into motion (http://www.floridaheart.org/pgzmotion/). Our laboratory has shown that pGz increases pulsatile shear stress on the vascular endothelium that increases release of eNO, prostaglandins, tissue plasminogen activator (t-PA), and adrenomedullin into the circulation. These substances are cardio-, neuro and cytoprotective. Pulsatile shear stress induced by pGz elicits a genomic response of up-regulation of eNOS in EC as well as nNOS in EC and cardiomyocytes. Using models of whole body ischemia reperfusion injury such as cardiac arrest, our laboratory has shown that pGz initiated one hour prior to cardiac arrest (preconditioning) decreases post resuscitation myocardial stunning and arrhythmias, and reduces biochemical indices of myocardial tissue injury and inflammation. pGz serves as a delayed postconditioning strategy after asphyxial cardiac arrest that diminishes myocardial stunning, tissue damage and inflammation. In another application of pGz, Martinez et al utilized postconditioning in a rodent model of ischemic stroke. This significantly decreased cerebral infarct size compared to control animals who did not receive pGz. The per-conditioning or conditioning potential of pGz has also been investigated. pGz when used as a method of resuscitation from ventricular fibrillation or asphyxial cardiac arrest decreased myocardial stunning, ameliorates reperfusion injury to a much greater extent than closed chest cardiac massage. Further, pGz treated survivors had excellent neurological outcomes. In contrast to pharmacologic strategies where drug distribution may be impaired in poorly perfused areas, pulsatile shear stress during pGz is independent of perfusion limitations and acts on the entire vasculature. Therefore, it allows wide availability of endothelial derived cytoprotective substances. The investigations that have cited in this paper have been conducted in large animal models of cardiac arrest and confirmatory studies in humans with cardiac arrest are sorely needed. pGz is a safe non-invasive intervention which is well tolerated in healthy humans and diseased patients. Also, pGz has the potential as complementary to pharmacologic or other interventions to improve microvasculature blood flow. This should aid in better distribution of drug availability.

**Conclusions**

The endothelium and in particular the microvasculature is adversely affected by I/R injury. Therapeutic interventions targeted to the endothelium which can ameliorate or reverse such injury are vitally important. Pharmacologic, gene based therapies and whole body periodic acceleration (pGz) have their basis in stimulating eNOS for its vasodilator, anti inflammatory, and antioxidant properties. Getting the right therapy/signal to the right place (microvasculature) at the right time (pre, per, post conditioning) will require tailoring the therapy to the specific I/R event. Understanding endothelial biology during ischemia reperfusion injury has potential to translate into successful life-saving, therapeutic strategies.

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