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ACUTE AND CHRONIC EFFECTS OF ENHANCED EXTERNAL COUNTERPULSATION ON HEMOSTATIC FACTORS IN CVD PATIENTS

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ACUTE AND CHRONIC EFFECTS OF ENHANCED EXTERNAL COUNTERPULSATION ON HEMOSTATIC FACTORS IN CVD PATIENTS

By

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ABSTRACT

ACUTE AND CHRONIC EFFECTS OF ENHANCED EXTERNAL COUNTERPULSATION ON HEMOSTATIC FACTORS IN CVD PATIENTS

By

Adam M. Coughlin

Enhanced external counterpulsation (EECP) has been shown to reduce angina and improve exercise tolerance. However, the fibrinolytic responses of treatment have yet to be determined. **Purpose:** Acute (pre and post one session) and Chronic (35 sessions at 60 minutes) effects of EECP on tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1) were evaluated. Additional chronic measurements of d-dimer and thrombin-antithrombin (TAT) were also examined. **Methods:** Twelve (four females and eight males) cardiac patients (age 69 ± 8 years, height 173 ± 9 cm, weight 87 ± 15 kg) referred to EECP therapy underwent traditional seven-week, five days/week, one hour/day course EECP therapy. Venous blood samples were drawn into an acidified citrate solution before the first week of therapy and upon completion of the course of treatment. Acute phase blood draws were performed prior to and immediately following a single treatment. Platelet-poor plasma from these samples was used to determine tPA activity, tPA antigen, PAI-1 activity, d-dimer, and TAT. **Results:** There was a significant difference in the acute tPA activity (pre = 0.79 ± 0.57 IU/ml, post = 1.00 ± 0.57 IU/ml, p = 0.012) and PAI activity (pre = 26.48 ± 42.63 IU/ml, post = 19.65 ± 33.15 IU/ml, p = 0.027) responses for a single EECP session. Acute tPA antigen response was not significant (pre = 10.18 ± 2.38 ng/ml, post = 9.99 ± 2.2 ng/ml, p = 0.523). Chronically, there were

no differences in tPA activity (pre = 0.79 ± 0.46 IU/ml, post = 0.65 ± 0.40 IU/ml, p = 0.061), tPA antigen (pre = 10.41 ± 2.67 ng/ml, post = 10.38 ± 2.60 ng/ml, p = 0.946), PAI activity (pre = 19.15 ± 20.41 IU/ml, post = 20.11 ± 18.14 IU/ml, p = 0.855), or d-dimer (pre = 574.9 ± 535.7 ng/ml, post = 462.8 ± 280.2 ng/ml, p = 0.3) following a seven-week course of EECP therapy. There was a nonsignificant decrease in TAT (pre = 1.71 ± 0.60 ug/L, post = 2.49 ± 1.49 ug/L, p = 0.08) following EECP therapy. **Conclusion:** These data suggest that EECP therapy acutely improves the fibrinolytic profile of cardiac patients by increasing tPA activity and decreasing PAI-1 activity. Chronically, coagulation appears to be inhibited, represented by a decreased TAT and unchanged d-dimer.

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Data displayed as mean ± standard deviation. *bpm* = beats per minute, *SBP* = systolic blood pressure, *DBP* = diastolic blood pressure
p value is reflective of chronic pre values compared to chronic post values.
*-Post-therapy value significantly different than pre-therapy value ($P < 0.05$)

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LIST OF ABBREVIATIONS

ANP	atrial natriuretic peptide
BNP	brain natriuretic peptide
CABG	coronary artery bypass graft
CAD	coronary artery disease
CCS	Canadian Cardiovascular Society
CDV	cardiovascular disease
CHF	coronary heart failure
DA	diastolic augmentation
DIC	disseminated intravascular coagulation
DVT	deep vein thrombosis
ED	erectile dysfunction
EECP	enhanced external counterpulsation
EF	ejection fraction
IEPR	International EECP Patient Registry
IPC	intermittent pneumatic compression
LVD	left ventricular dysfunction
NO	nitric oxide
PAI-1	plasminogen activator inhibitor
PCI	percutaneous coronary intervention
PEECH	prospective evaluation of EECP in heart failure
SU	systolic unloading
TAT	thrombin-antithrombin
TF	tissue factor
TFPI	tissue factor pathway inhibitor
tPA	tissue plasminogen activator
VEGF	vascular endothelial growth factor
vWF	von Willebrand factor

Chapter One

Introduction

Cardiovascular disease (CVD) accounts for 38.4% of all deaths and claims more lives each year than the next five leading causes of death combined (1). CVD is a progressive disease, ultimately manifesting as one of several acute ischemic events, including stroke, myocardial infarction, and transient cerebrovascular and cardiovascular ischemic attacks. Therefore, increasing knowledge about factors predisposing individuals to acute ischemic events is warranted.

Typically, hemorrhage or ulceration of atherosclerotic plaque initiates an acute ischemic event. Clot formation inside the ulcerated plaque or in the atherosclerotic vessel occludes the vessel and the ischemic event occurs. However, plaque ruptures have been observed in autopsy studies of individuals without history of ischemic events (13, 47), suggesting that plaque rupture does not always result in occlusive clot formation, or thrombus. Therefore, the coagulative state of the blood likely determines the extent of thrombus formation. In support of this, fibrinolysis (the capacity to dissolve excessive or inappropriate clot) (11, 22, 28, 40, 48, 55, 68) is decreased in patients with CVD. Furthermore, a decreased fibrinolytic potential is associated with risk of future ischemic events in coronary artery disease (CAD) patients (68).

The main enzyme in fibrinolysis is tissue plasminogen activator (tPA), which is responsible for the conversion of plasminogen into plasmin. The

activated plasmin is then able to dissolve fibrin clots into fibrin dimer proteins. The main circulating inhibitor of tPA is plasminogen activator inhibitor (PAI-1) which, when bound to tPA, forms an inactive tPA•PAI-1 complex. Therefore, decreased tPA activity and increased PAI-1 are indicative of impaired fibrinolysis, and are associated with CVD (14, 28, 55, 59, 71), CAD (50, 54), myocardial ischemia (11, 12, 22, 68), stroke (26, 38), and mortality (25).

Enhanced external counterpulsation (EECP) is a noninvasive, outpatient treatment for patients with chronic angina who are not candidates for conventional forms of revascularization, or for patients who refuse invasive treatments. EECP is an EKG-driven, computer-controlled pneumatic pump that inflates and deflates a series of inflatable cuffs surrounding the lower extremities. During diastole, sequential filling of the paired cuffs surrounding the calves, lower, and upper thighs inflate to a therapeutic pressure of 260-280 mmHg within milliseconds. Deflation of the three, paired cuffs occur simultaneously prior to systole. Inflation and deflation are controlled by cardiac cycle events monitored by the EKG, thus increasing venous return without a concomitant increase in total peripheral resistance. In fact, an acute drop in systolic pressure occurs during treatment sessions (45). A typical course of treatment usually lasts for seven weeks, consisting of 35 sixty minutes sessions.

EECP technology has existed for four decades, but due to associated costs and lagging technology, did not become popular until recently. Since that time, research has shown that EECP reduces angina (39, 57, 66) and nitrate use (39, 57), increases exercise tolerance (60, 66, 70), enhances quality of life (39,

60), eliminates or reduces time to exercise induced ST-segment depression (66), reduces myocardial ischemia (66, 70), improves left ventricular diastolic filling (70), and improves endothelial function (7, 57). Significant portions of these beneficial effects are associated with transiently increased shear stress and improved vasoreactivity. Shear stress increases nitric oxide (NO) (56), which subsequently could affect tPA and PAI-1 release from endothelial cells. Furthermore, improved endothelial function realized from EECP therapy could impact fibrinolysis, as endothelial cells are the major sites of tPA synthesis and release. However, despite the possible effect of EECP treatment on fibrinolysis, few data exist on the hemostatic impact of EECP therapy.

Therefore, the specific aims of this study are to determine: 1) the thrombotic and fibrinolytic adaptations to 35 sessions of EECP and 2) the acute fibrinolytic responses to one 60-minute session of EECP. It is hypothesized that beneficial fibrinolytic responses and adaptations will occur, reflected by: increased tPA activity and decreased PAI-1 activity. Furthermore, it is hypothesized that chronic EECP therapy will decrease thrombin-antithrombin (TAT), a marker of thrombosis.

Chapter Two

Review of Literature

Overview of coagulation

Hemostasis is the physiological response to injury where bleeding is stopped by the interaction of the severed blood vessel, platelets and soluble coagulation factors to form an insoluble fibrin clot. Fibrin clots are initiated by de-encryption of the factor VII receptor: tissue factor (TF) and the subsequent formation of TF, initiating the coagulation pathway. Factor VII complex triggered by a series of enzymatic reactions results in the ultimate release of thrombin from prothrombin. Thrombin, the only coagulant enzyme in mammals, activates platelets, converts soluble fibrinogen to insoluble fibrin and, by positive feedback, produces more thrombin from its precursor prothrombin. Thrombin also initiates the process of clot lysis, or fibrinolysis. A thrombus is a blood clot that forms in circulating blood. Thrombi form by the exact mechanism as hemostatic clots when TF is de-encrypted following endothelial injury by atheroma plaque or attack by activated complement factors. Both thrombi and hemostatic clots impair circulating blood and are eliminated by fibrinolysis (52).
Thrombin-antithrombin (TAT) complexes are formed when antithrombin III is irreversibly bound to the active site of thrombin. The clinical significance of the determination of TAT complex is in the diagnosis of thrombotic events. Patients predisposed to thrombosis and disseminated intravascular coagulation (DIC) are found to have elevated TAT, which increases as a function of disease.

Overview of fibrinolysis

When thrombin is activated and a crosslinked fibrin clot is formed, plasmin immediately begins to degrade the fibrin network. Plasmin is activated from its zymogen (plasminogen) by tissue-type plasminogen activator (tPA). tPA activation of plasminogen can be prevented by binding in a one-to-one complex with the main circulating tPA inhibitor, plasminogen activator inhibitor (PAI-1). The binding of tPA to PAI-1 forms an irreversible enzymatic inhibition and is cleared from circulation at a faster rate than either free tPA or PAI-1.

Sites of synthesis, release and activation of coagulation

Most factors involved in coagulation are synthesized in, and released from, the liver (fibrinogen, prothrombin, factors V, VII, IX, X, XI, and XIII), unknown (factor VIII and XII), or in megakaryocytes (factor XIII). These factors are activated, as previously stated, in the presence of thrombin. Tissue factor protein inhibitor (TFPI) and antithrombin neutralize factor VIIa, only if bound to tissue factor (44). Endothelial cells contain virtually no tissue factor activity under normal conditions. However, under certain circumstances endothelial cells can develop a high level of TF activity in the presence of thrombin, fibrin (10), shear stress (37), and hypoxia (49).

Sites of synthesis, release and activation of fibrinolysis

Endothelial cells have been shown to be the main producers and sites of tPA release into the blood (44). tPA circulates in the blood until inactivated by

PAI-1. Unbound tPA is active in converting plasminogen into the active plasmin for normal fibrinolysis. However in the presence of fibrin, tPA increases the converting activity 1000-fold, thereby increasing activity where it is needed most (44).

PAI-1 is synthesized and released from alpha granules during platelet activation and is also synthesized and released from endothelial cells, fibrosarcoma cells, hepatocytes, and in the placenta (44). Synthesis, as well as release, is affected by thrombin and other inflammatory response elements (44).

Coagulation and fibrinolytic status in CVD patients

Although asymptomatic coronary artery plaques are present in most Westernized populations, their progression determines cardiovascular disease (CVD) progression. Lipid depositions beyond the tunica intima, specifically into the subendothelial layer, invoke an inflammatory response. This causes an overall growth of the plaque, leading to necrosis and an advanced lesion. Given enough time the lesion becomes calcified and brittle. Ruptured lesions activate the TF pathway of coagulation and without adequate fibrinolytic profile, symptomatic occlusion occurs. Thus, hemostasis is clinically significant with respect to CVD outcomes. Patients with CVD have a heightened potential for coagulation and a diminished fibrinolytic capacity. This is marked by high levels of TAT and d-dimer, as well as decreased tPA activity with concomitant increases in both tPA antigen and PAI-1 activity. This undesirable profile is

rarely identified until symptoms develop as a consequence of chronic pro-thrombotic and/or anti-fibrinolytic activity coupled with endothelial dysfunction.

Treatments for angina

Two treatment goals for stable angina are: 1) to prolong the life of the patient and to reduce the incidence of adverse outcomes, and 2) to reduce the frequency and intensity of angina symptoms and increase the duration of angina-free exercise (67). Most treatment options address both goals and subsequently address comorbid conditions, lifestyle modifications, and risk factor modifications. A 1999 review article lists the following as current treatment options for stable angina: cigarette smoking cessation, aspirin, aspirin alternatives, statins for dyslipidemia, treatment of hypertension, hormone replacement therapy in postmenopausal women, revascularization procedures, angiotensin-converting enzyme inhibitors, antianginal drugs such as β- blockers, nitrates, and calcium channel blockers, controlling diabetes mellitus, antioxidants, enhanced external counterpulsation (EECP), spinal cord stimulation, and transcutaneous electrical nerve stimulation (67).

In a 2002 review article, Kim et al. (29) suggest that conventional approaches to treating angina should restore the imbalance between coronary blood flow and myocardial oxygen demand, starting with medications, lifestyle modifications, revascularization techniques, and atherectomy techniques. The commonly prescribed medical therapies between the two review articles are similar; however, the recent article introduces some additional treatment options

that have emerged, including: EECP, laser revascularization, percutaneous in situ coronary venous arterialization, chelation therapy, and even heart transplant. In all cases the treatment strategy is focused on either decreasing oxygen demand or increasing the supply of blood, or both.

Treatment strategies are grouped into one of three categories: pharmaceutical, noninvasive nonpharmacological, and invasive therapies. Because lifestyle modifications are seldom sufficient to reverse the pathology associated with diseased arteries, pharmaceutical agents are often employed to help correct the disease state. However, medication often fails to address every symptom associated with angina. Because of this, as well as drug interactions and risk of overmedicating the patient, progression from pharmaceutical agents is necessary. Therefore, the need for effective, preferably noninvasive and cost-conscious alternatives have given rise to newer low-risk, anti-ischemic therapies as additional treatment options.

A 2002 review of treatment options and the future use of EECP (24) states that the existing algorithm for symptomatic coronary artery disease is pharmacological intervention followed by coronary artery bypass graft and/or percutaneous catheter intervention, followed by EECP or other tertiary option. The author suggests that future algorithms include EECP as an additional secondary option, or the next step following the failure of pharmacological agents. Additionally, the fact that bypass or angioplasty is contraindicated for patients with left ventricular dysfunction suggests a higher priority for EECP.

Furthermore, EECP is effective regardless of previous revascularization and could actually be considered a first choice over invasive revascularization (19).

Mechanics and recommendations of EECP

Vasomedical, Inc. was granted FDA clearance to market their EECP – MC2 model for prescription use in February 1995. The device was indicated for use as a non-invasive treatment of patients with stable or unstable angina pectoris, acute myocardial infarction, or cardiogenic shock (15). In June of 2002, Vasomedical, Inc. was granted the addition of congestive heart failure, currently making the intended use for treating patients with stable or unstable angina, congestive heart failure, acute myocardial infarction, or cardiogenic shock (69).

The most current EECP device is comprised of three major components, a control console, a treatment table, and a patient cuff set. The control console houses the air compressor and reservoir, a signal module panel, a power module, a microprocessor with touch screen/keyboard interface, data storage device and printer, and components for acquiring and processing ECG and finger plethysmograph signals. The treatment table accommodates a motorized lifting mechanism, mattress, and the pneumatic circuit valve assembly. The motorized lifting mechanism is used to move the mattress up and down, providing a convenient height for patient and operator use. The valve assembly consists of three pairs of inflation/deflation valves that open and close on command to inflate or deflate the patient cuff set with air. The valve assembly is connected to the air

compressor and reservoir components in the control console via connecting air hoses.

External pressure is applied via the patient cuff set to the lower extremities of the patient in synchronization with the cardiac cycle, such that the cuffs sequentially compress vascular beds in the calves, lower thighs, and upper thigh/buttocks during diastole. This sequential application of external pressure forces blood back to the heart, increasing coronary perfusion pressure and blood flow (diastolic augmentation), as well as increasing venous return. Immediately before the heart begins to eject blood during the next systolic phase, the cuffs are deflated rapidly and all externally applied pressure is eliminated. The vasculature in the lower extremities reconforms and is able to receive the output of the heart with lessened resistance, thereby reducing systolic pressure and the workload of the heart (decreased afterload).

The usual EECP prescription for treatment of angina is one to two hours per session of five sessions per week for seven weeks, totaling 35 hours of treatment. Information available at the Vasomedical Inc. website (http://www.eecp.com/) states that effective July 1, 1999, the Health Care Financing Administration (HCFA) provided coverage for EECP therapy to Medicare patients who have been diagnosed with disabling angina. These patients, in the opinion of a cardiologist or cardiothoracic surgeon, are not readily amenable to surgical intervention because: 1) their condition is inoperable, or at high risk of operative complications or post-operative failure, 2) their coronary anatomy is not readily amenable to such procedures, or 3) they have comorbid

states, which create excessive risk. Additionally, private insurance carriers make their own determinations as to what services are covered and the level of reimbursement. Over 140 insurance carriers pay for EECP therapy on a case-by-case basis, with an increasing number offering coverage on a blanket basis. Reimbursement has been granted due to the clinical trials and subsequent research illustrating multiple benefits due to treatment.

Proposed mechanisms of action

The mechanistic principle of EECP therapy is to mechanically increase diastolic perfusion pressure, and venous return, and decreasing cardiac afterload. Sequential inflation of the cuffs produces an aortic counterpulsation in addition to increasing venous return. The aortic counterpulsation increases diastolic blood volume and pressure (DA) in the aorta. The release of pressure from the cuffs decreases afterload (SU) and results in an increased cardiac output at lower systolic pressures. DA and SU, and the DA/SU ratio, can be calculated from a graphical waveform analysis via finger plethysmography. This mechanical experience is hypothesized to be associated with the amount of diastolic augmentation (DA) and systolic unloading (SU) attained during EECP treatment. This explanation was evaluated by Suresh, et al. (65). The authors postulated that the acute hemodynamic effectiveness of EECP is proportional to the ability to increase DA and decrease SU, and that these measurable values can be simply expressed as a ratio of DA to SU (as DA/SU). The specific aims of the study were to assess: 1) the relationship of DA/SU during EECP, 2) changes

in systolic and diastolic descending aortic flow at rest and during EECP and, 3) the optimal DA/SU range to maximize antegrade systolic and retrograde augmented diastolic aortic flow.

Fifteen subjects (age 54 years, range 38-66, 14 men) were enrolled and studied during a single bout of EECP. Changes in retrograde diastolic volume, cardiac output, and aortic retrograde to antegrade flow ratio were graphed with respect to the DA/SU ratio. Graphical evidence illustrates a linear relationship of all parameters and DA/SU ratio at values of zero to 1.5. However, these linear relationships plateaued between a ratio of 1.5 to 2.6. The authors did note that the variability in individual responses to changes in DA/SU resulted in some patients only reaching a maximal ratio of 1.2-2.0.

The authors drew four conclusions: 1) EECP has marked effects on the magnitude of DA and SU; 2) the hemodynamic effects of DA and SU are dependent on the magnitude of the applied external cuff pressure; 3) there is a positive linear relationship between the noninvasively assessed EECP DA/SU ratio and cardiac output, the diastolic time velocity integral (retrograde diastolic volume), and the diastolic to systolic time velocity integral ratio (aortic retrograde to antegrade flow ratio) in the descending aorta; and 4) an EECP DA/SU ratio that ranges from 1.5 – 2.0 maximizes the hemodynamic effects of EECP and minimizes the possibility of cuff barotrauma.

Michaels, et al. (46), hypothesized that patients undergoing EECP who are able to achieve higher DA ratios may derive greater clinical benefit. For this study, the authors identified 1,004 patients that had undergone 35 hours of

treatment and had six-month follow-up data. Prior to analysis, patients were dichotomized into those receiving treatment with DA ratios ≥ 1.5 and those with DA ratios < 1.5. Thirty-seven percent of the patients were able to generate DA ratios ≥ 1.5 on the last day of EECP therapy. There were no significant differences between the two groups post-EECP in terms of events during course of treatment, however, those with a DA ratio ≥ 1.5 had more reductions in Canadian Cardiovascular Society (CCS) angina class, fewer patients using nitroglycerin, and a higher reported quality of life score. Six-month follow-up data showed patients with a DA ratio ≥ 1.5 experienced less unstable angina and CHF, more percutaneous coronary intervention (PCI), additional improvements in CCS angina class, and more patients reporting a good to excellent quality of life score. This evidence supports the claim that a higher DA ratio, particularly one greater than 1.5 is important for achieving maximal clinical benefits from EECP. Interestingly, most patients did not achieve a DA ratio above 1.5, in spite of receiving a maximal therapeutic cuff pressure. Follow-up data analysis showed that patients receiving lower DA ratios were older, female, hypertensive, hyperlipidemic, diagnosed with diabetes mellitus, positive for family history, diagnosed with noncardiac vascular disease, and smokers.

Lakshmi, et al. (30) did not use the DA ratio cut-point of 1.5, but grouped the patients into quartiles. Patients were divided according to whether their DA was above or below the median value at the first and last days of EECP therapy. Utilizing the International EECP Patient Registry (IEPR), 2,486 patients were identified for analysis. The median value for the initial DA was 0.7 and the final

DA was 1.0. Patients were categorized as being low-low (n = 1,009) if their DA was below 0.7 during the first EECP treatment and below 1.0 for the last EECP treatment. Low-high (n = 281) patients had initial DA less than 0.7 and their last DA was above 1.0 at the last EECP treatment, high-low (n = 250) was a DA above 0.7 initially and a DA below 1.0 at the last EECP treatment, and high-high (n = 946) patients were identified as having a DA above 0.7 initially and above 1.0 at the last EECP treatment. Baseline characteristics showed that the low-low group was older, contained fewer women, had a longer history of coronary disease, and was more likely to have a history of hypertension, diabetes, noncardiac vascular disease, and current smoking. The high-high group contained more candidates for revascularization and had the lowest baseline angina class. All patients completed at least 35 hours of EECP therapy. Immediately after EECP, low-high patients had the greatest reduction in CCS angina class, with high-low patients having the least reduction. These data suggest that there is a mechanistic role, via vascular tone, in the relationship between DA and clinical benefits with EECP.

Despite research elucidating the benefits of EECP treatment, the mechanism(s) of action responsible for these benefits remain unclear. In 2001 Masuda, et al. (42) reported that EECP improves coronary endothelial function and decreases peripheral resistance and heart rate response to exercise, but no proposed mechanism of action. A recent review (17) highlighted two mechanistic hypotheses for the efficacy of EECP therapy. The first hypothesis is that the effects of EECP are due to enhanced diastolic flow, which causes the release of

an angiogenic growth factor, this initiating the development of new collateral blood vessels. In support of this hypothesis, one published abstract (27) and one peer-reviewed study (43) reported an increase in circulating angiogenic growth factors following EECP therapy.

The second hypothesis attributes EECP benefits to improvements in vascular reactivity. Specifically, a decrease in endothelin (73), a potent vasoconstrictor, and an increases in nitric oxide (51, 73), a potent vasodilator, would improve vascular reactivity and coronary flow. Additional evidence suggests that EECP therapy decrease markers for left ventricular stress, such as atrial and brain natriuretic factors (27, 42). An additional hypothesis is that 35 hours of EECP treatment results in a transient decrease in myocardial work. This myocardial rest could normalize neurohumoral signals, thus improving arterial compliance and arteriolar reactivity (17), and producing a "training effect" of treatment.

Physiological evidence of adaptations to EECP treatment

Effects on myocardial perfusion

EECP improves survival in patients with cardiogenic shock (62) and reduces mortality rates following myocardial infarction (2). Lawson et al. published the seminal paper on the effect of EECP on myocardial perfusion (32). Eighteen patients with chronic, stable angina and exertional ischemia were included in their study. The patients underwent thallium stress tests before and after EECP treatment. All 18 patients reported substantial improvements in

anginal symptoms following EECP treatment, with 16 reporting complete relief during activities of daily living. The thallium-201 stress testing showed complete resolution of ischemic defects in 12 patients, a decrease in the area of ischemia in two patients, and four patients reported no change. Maximal exercise duration significantly increased, by a mean of 1.46 minutes, with no change in double product. The authors noted that the EECP was well tolerated, with no subject withdrawals, and no reported complications.

Three-year follow-up data on the fourteen subjects that had improvements in the thallium-201 stress test were available on ten subjects. All follow-up tests demonstrated a sustained reduction in angina with eight continuing to show improvements in myocardial perfusion (35). During the time of the initial and follow-up studies, patients reported fewer and less severe anginal episodes and an improved exercise tolerance after EECP treatment. This prompted a study to specifically investigate improvements in exercise tolerance and to access exercise hemodynamics due to EECP treatment (33). Twenty-seven patients (26 men) conducted the same protocol used in the prior study (32). Perfusion, via radionucleotide stress test, improved in 21 patients and remained unchanged in the other 6 patients. Maximal exercise duration improved 1.67 minutes, with 22 patients (81%) experiencing longer test durations. Maximal heart rate, blood pressure, and double product did not differ in the 27 patients. The 21 patients who improved perfusion following EECP showed an even greater increase in maximal exercise duration (average of 1.9 minutes) and a statistically significant increase in peak heart rate by over ten beats per minute. In contrast, the six

patients who did not improve perfusion did not increase peak exercise duration or peak heart rate. This study further confirmed that EECP improves myocardial perfusion and decreases vascular resistance.

In addition to these two seminal studies, another study (35) evaluated the effects of EECP on myocardial perfusion, using the same 18 patients reported on in the previous study (32). This Lawson, et al. also published a study comparing responses to EECP in 60 patients who were previously revascularized to patients that were not (31). Considering the list of authors and the reported methodology, it is plausible that these four studies utilized the same base of subjects. Therefore, the initial five years of publishing on this topic may be based on only 60 subjects.

Effects on angina in coronary artery disease

The organization of the IEPR and FDA approval for the EECP machine in 1995 sparked an outburst of publications. The IEPR was organized in 1998 (6) and a multicenter study was initiated in 1995 (5). The IEPR was initiated to document both safety and efficacy of EECP and long-term outcomes. All registry patients are treated with EECP equipment (Vasomedical Inc., Westbury NY) as described earlier. Criteria for entry are informed consent and completion of at least one hour of EECP treatment. Prior to the treatment, a one-page form is completed describing demographics, medical history, disease characteristics, and symptoms. Upon completion of the last hour of treatment, another single page is filled out regarding the length of treatment, the degree of diastolic

augmentation, untoward clinical events, and symptomatology. Follow-up via telephone interviews occurs six, 12, 24, and 36 months after completion of therapy. Data is obtained regarding clinical events, hospitalizations, anginal status, and quality of life (6).

The MUlticenter STudy of Enhanced External CounterPulsation (MUST-EECP) was published in 1999 (5) as the first prospective, randomized, blinded, placebo-controlled trail designed to evaluate EECP patients with angina and documented coronary artery disease (CAD). EECP treatment effects were compared using exercise treadmill test data and anginal symptoms. Seven US medical centers produced over 500 patients who were used to select 139 eligible patients for analysis. The 139 patients were randomized into EECP treatment (n = 72) or sham (n = 67) groups following a medical history, physical examination, and a baseline exercise treadmill test. Once randomized, all patients underwent 35 hours of treatment. The sham group received a subdiastolic pressure of 75 mmHg. Initial exercise treadmill testing was completed within four weeks of treatment initiation. Post-treatment, exercise treadmill testing was completed within a week of completing the 35^{th} treatment session. Years of angina (9yrs vs 5yrs) and previous MI (56% vs 41%) were statistically different in the EECP compared to the sham group.

Resulting data from the treadmill test showed an increase in exercise duration in both groups from pre to post but no difference when comparing treatment to sham patients ($p < 0.31$). Time to $\geq$ 1-mm ST-segment depression did not increase in the sham group, but did in the group when comparing pre to

post treatment. When comparing all patients who started receiving treatment, there was a non-significant trend in reduction of angina counts for the EECP group compared to the sham ($p < 0.09$). When analyzing patients who completed 35 hours of treatment versus those that completed less than 35 hours, there was a significant difference between the groups ($p < 0.035$). This suggests that there is either a certain number of treatments that must be conducted before reductions in angina counts are realized or that the patients who did not notice differences in angina counts removed themselves. Nitroglycerin use between the EECP and sham groups was not different. Significantly more EECP patients reported adverse events than sham patients, both overall (non-device and device related), 55% vs. 26%, respectively, and device related. The device-related experiences included paresthesia, edema, skin abrasion, bruising and blistering, and leg and back pain, with paresthesia being the only one not statistically significant between EECP and sham groups. For exercise duration, EECP and sham groups reported 432 seconds versus 426 seconds, respectively. EECP improved exercise duration by an average of 54 seconds, while the sham group improved 32 seconds. While the same argument remains for time to ST-segment decrease, results were sufficiently robust to show significance without a proper pre-EECP assessment.

Patients who have undergone surgical revascularization may respond differently to EECP that those using EECP. In 1998 Lawson, et al. (31) compared responsiveness to EECP in 60 patients who had undergone coronary artery bypass grafting to those who had received no form of surgical

revascularization. The investigators also took into account single-, double-, and triple-vessel disease states within each group. All subjects reported a decrease in anginal symptoms (frequency, severity, ease of precipitation, and duration of episodes) as well as improvements in angina-limited exercise tolerance. A similar percentage of patients showed improvements on their post-EECP radionucleotide stress tests in revascularized and unrevascularized groups (80% and 88% of the patients showing perfusion improvements, respectively). However, when level of vessel disease was dichotomized into one- and two-vessel versus three-vessel groups, percentage of patients who underwent revascularization (80% of the patients improved their perfusion) illustrated marked improvements compared to patients that were unrevascularized (20% of the patients improved their perfusion). These data suggest that revascularization is only a consideration of EECP if the patient has more than two-vessel occlusion, suggesting that myocardial perfusion will increase if there is sufficient healthy vasculature.

Fitzgerald, et al. (19) compared the effects of EECP therapy on patients who underwent coronary artery bypass graft (CABG) versus non-revascularized EECP patients. The authors hypothesized that the post-CABG patients would show greater improvements due to the limited recruitment and development of collaterals in the non-revascularized EECP patients. Using data collected up to September 2000 from the IEPR, 95% (n = 4,239) had undergone some sort of revascularization and 5% (n = 215) had not. Upon completion of the treatment, there was a reduction in CCS functional angina class of over 70% in both groups,

a marked reduction in angina episodes per week, and a reduction in the nitroglycerin use. Six-month follow up data on almost 80% of the initial participants demonstrated further improvement in angina class. Angina episodes per week were the same or less than initial post-EECP values in 79.4% of the previously revascularized and 89% of the comparison group. Nitroglycerin use increased more in the previously revascularized group at the six-month post-EECP time-point. These large-scale results suggest the possibility of using EECP as a first choice over invasive revascularization based on the similar adaptations to EECP treatment. However, the study design was not set up to compare degree of vessel disease extent as per the Lawson et al. study (31).

Barsness, et al. (6) considered the effects of EECP in the first 978 patients in the IEPR. The authors reported on the initial level of vessel disease (none, single, double, or triple). Similar to the aforementioned articles there were no reported findings with respect to the initial level of vessel disease. Only Lawson, et al. (34) investigated EECP benefits in patients initially presenting with multivessel disease and reported the responses based on level of vessel disease (one, two, or three vessel residual disease). Fifty patients (mean age 61 years, range 45-75 years, 46 men) with chronic stable angina, with angiographic coronary disease (requiring > 70% stenosis in a major vessel), and exercise-induced reversible radionucleotide perfusion defect(s) were included in the study. Radionucleotide stress testing was performed to peak exercise tolerance at baseline, followed by seven weeks of EECP (one hour a day, five days per week). Within one week of cessation of therapy another radionucleotide stress

test was conducted to the same cardiac workload as the baseline test. While there was a reported decrease in angina symptoms following EECP, there was also a statistically significant improvement in radionucleotide stress perfusion imaging after EECP ($p < 0.001$). Analysis showed an inverse relationship between change in perfusion and the extent of coronary disease ($p < 0.01$). Ninety-five percent of single vessel disease patients, 90% of those with double vessel disease, and 42% who showed triple vessel disease improved perfusion from pre to post EECP. These data suggests that having no more than two vessels occluded increases the likelihood of increasing the incidence and degree of perfusion. Thus, patients with three or more occluded vessels may be better served if some sort of revascularization is performed prior to EECP.

While Barsness, et al. (6) did not analyze specifically for degree of vessel disease they grouped patients based on whether they were candidates for revascularization. Baseline characteristics between the two groups were similar with respect to age, gender, race, previous EECP treatment, and risk factors such as family history of CAD, diabetes, hypertension, hyperlipidemia, and past or present smoking. Patients who were not candidates for revascularization had more years of CAD history, and higher percentages of percutaneous coronary intervention, coronary artery bypass grafting, prior myocardial infarction, congestive heart failure, and noncardiac vascular disease. Disease status of the two groups differed with the non-candidate patients exhibiting higher CCS classification, higher incidence of unstable angina, a greater degree of left ventricular ejection fraction dysfunction, more reported episodes of angina per

week, and a majority of the triple vessel disease. Over 84% of the patients finished the 37 hours of treatment. There was a significant difference reported in the mean diastolic augmentation between the two groups for the last hour of treatment. All patients showed reductions in CCS angina class (80% compared to 84%, for non-candidates and candidates respectively). Both groups exhibited a decrease in nitroglycerin use, decreased angina episodes per week, and improved quality of life. This study shows that EECP therapy is safe and effective for the reduction of angina and related symptoms in a heterogeneous group of patients, regardless of alternative revascularization options. While this finding is potentially promising for patients wishing to undergo noninvasive treatment, neither a control group nor long-term follow-up was utilized in the study.

Effects on congestive heart failure

Patients undergoing EECP have also been dichotomized with respect to history of congestive heart failure (CHF). It could be argued that due to having more extensive coronary and vascular disease, CHF patients would show fewer benefits from EECP, and a faster return to baseline once treatment has stopped. Lawson, et al. (36) sought to analyze the response of EECP in patients with CHF versus patients without. Using IEPR data, 1,957 patients were identified as having six-month follow-up data available. Twenty-eight percent, or 548 patients, of this group had a medical history of diagnosed CHF at baseline. Patients without CHF had statistically fewer years since CAD diagnosis, higher left

ventricular ejection fraction, a higher percentage of men, younger age, more candidates for PCI or CABG, and lower percentages of PCI, CABG, MI's, family history of CAD, multivessel CAD, diabetes, hypertension, and noncardiac vascular disease. The only similar characteristics were percentages of hyperlipidemia and past and/or present smoking. Seventy-five percent of patients without CHF saw a reduction in angina class versus 68% in patients without CHF. Patients without CHF reported exacerbation of CHF and musculoskeletal discomfort less frequently than those patients with CHF. At the six-month follow-up, both groups reported continued improvements in CCS angina class. Of the CHF patients without a MACE, 82% reported that their angina was the same or less than when they finished EECP. There were adverse events reported within the six-month follow-up, but the authors noted that, due to the severity of the disease, these events were within expectations. The authors also noticed differences in MACE and CHF exacerbations between patients with CHF dichotomized by a LVEF of 35% both during the treatment and at the six-month follow up. Therefore, EECP was as equally effective in treating patients with and without CHF, both during treatment and follow-up.

Soran, et al. (60) studied 26 patients, 7 with idiopathic dilated cardiomyopathy and 19 with ischemic cardiomyopathy. Differences between the idiopathic and ischemic groups included age (41 vs. 64 years, respectively), LVEF (19 vs. 26%, respectively), angina (0 vs. 62%, respectively), and a higher percent of patients taking digoxin and carvedilol in the idiopathic group. Patients were followed for a period of six months, with data points recorded at one week,

three months, and six months following the last EECP treatment session. In the 23 patients measured at one-week post-EECP, there was a 0.98 ml/min/kg increase in peak oxygen uptake from baseline (14.99 to 15.98 ml/min/kg, 7.45% increase, $p = 0.05$) and an increase in mean exercise duration of 105.33 seconds from baseline (627.63 to 732.96 seconds, 20.53% increase, $p < 0.001$). For the 19 patients with six-month follow-up data, the change in peak oxygen consumption, comparing baseline to six-month post EECP, continued to show an increase of over six ml/min/kg (14.78 to 18.41 ml/min/kg, $p < 0.001$) and a sustained increase in mean exercise duration of nearly 80 seconds compared to baseline (637.13 to 715.17 seconds, $p = 0.028$). The authors note that there were no between-group differences with respect to peak oxygen consumption or mean exercise duration. However, this could have been due to the four patients who were lost to follow up, which could possibly explain why peak oxygen increased from one-week post-EECP to six-month post-EECP, 15.98 to 18.41 ml/min/kg, respectively, with a corresponding decrease in mean exercise duration from the same time points (732.96 to 715.17 seconds). Quality of life was measured via the Minnesota Living With Heart Failure questionnaire (MLHFQ), which was administered at baseline, one-week post, and six-month post. In the 24 patients that completed the one-week post MLHFQ, there was a significant ($p < 0.01$) improvement between baseline and one-week post for total score, physical dimension, and emotional dimension. The 22 patients with six-month post data demonstrated a persistent improvement over baseline values, but only the emotional dimension remained significant ($p < 0.01$). Only one

subject experienced worsening of a preexisting arrhythmia, and there were no cases of a worsening heart condition during the treatment sessions of EECP.

Adverse events such as shin tenderness, skin abrasion, back and muscle pain, and swelling under knees were attributed to the treatment itself and other events classified as not related to treatment. In all there were 46 reported adverse events from 23 patients. Twenty-two events were reported during the 7 weeks of treatment, with 24 reported during the six-month follow-up. Although, eight patients reported events serious enough to lead to hospitalizations; none were reported as being related to the EECP device itself. Of the 22 events reported during the 7 weeks of treatment, ten occurred during EECP treatment session and the other 12 were reported between treatment sessions. Considering the expected outcomes of this type of disease, EECP was safe and well tolerated in patients who had stable heart failure and received no fluid overload. The authors noted a lack of a control group and a small sample size as limitations. However, the results indicate short and long-term benefits of EECP in patients with chronic stable heart failure, specifically: increased quality of life, exercise duration, and peak oxygen consumption. Furthermore, these adaptations persisted six months post EECP. These data suggest that EECP is safe and effective in CHF patients, with efficacy experienced up to six months post EECP.

Soran, et al. (61) investigated EECP in patients with CHF and left ventricular dysfunction (LVD). The investigators utilized IEPR data on 1402 patients that had ejection fraction (EF) data available and six-month follow up

data. The authors specifically examined the safety and efficacy of EECP for the relief of angina in patients with LVD, specifically those with a LVEF less than, or equal to, 35%. Comparisons were made between those with and without LVD who were enrolled into the IEPR by December of 1999. Of the 1402 patients with recorded LVEF data, 312 had EF less than 35% (mean EF was 28.6% ± 6.2%), with 1090 patients above 35% (mean EF was 52.1% ± 9.2%).

Baseline characteristics of the two groups were similar in terms of age, percentage of males, angina episodes per week, and risk factors. The non LVD group was significantly better in terms of medical history, CCS angina classification, nitroglycerin use, and multivessel disease, but had more candidates for both PCI and CABG. Eighty-six percent of the non LVD group and 79% of the LVD group completed therapy. Twice as many patients stopped treatment due to a clinical event in the LVD group (14.4%). However the percentage of patients who discontinued were the same, 7% in the non LVD versus 6.2% in the LVD group. While both groups exhibited improvements in CCS angina class, those without LVD decreased by one or more classes (76% of the patients, compared to 68% in the LVD group). Both groups showed a decrease in the angina episodes per week and the use of nitroglycerin. There were significantly more deaths in the non LVD group (3%) than the LVD group (1.6%), less unstable angina in the non LVD group (2%) than the LVD group (4.2%), and less exacerbation of CHF in the non LVD (1%) compared to LVD group (5.4%). For the six-month follow-up, significantly fewer deaths (2.2 vs. 9.3%), exacerbation of CHF (3.7 vs. 9.9%), percentages in CCS angina class III

or IV (16.2 vs. 27.3%), and percentages of patients using nitroglycerin (37.4 vs. 46.1%) were noted in the group without LVD compared to LVD patients. Of interest, MI (3+%), repeat EECP (11.5%), hospitalization (coronary, 12%, and/or noncoronary, 7%), and the percent of those experiencing no death, MI, PCI, CABG, and percent with improved or unchanged angina (~70%) were not different between the groups. Overall, these data suggest appropriate safety and efficacy regardless of presence of LVD. Although the benefits seen in the LVD group did not equal that of the healthier controls, the benefits were still present. Benefits for both groups remained at six months post, with expectedly more adverse events occurring in the LVD group.

Currently, Feldman, et al. (18), utilizes a controlled, randomized, single-blind, parallel-group, multicenter study involving 187 patients with symptomatic but stable heart failure and LVEF of < 35%. Patients are randomized into two groups, EECP treated and usual care. This Prospective Evaluation of EECP in Heart Failure (PEECH) study is specifically designed to evaluate EECP as an adjunctive therapy to guideline-mandated medical care. Patients are randomized and stratified by etiology of heart failure (ischemic or idiopathic), age, gender, treatment with an ACE-inhibitor or angiotensin II receptor blocker, and treatment with a beta-blocker. Patients assigned to the EECP group undergo a total of 35 hours of EECP treatment at one-hour increments for a total of seven to eight weeks. Exercise tolerance tests are performed and analyzed using a modified Naughton protocol. Quality of life measurements are measured using the MLHFQ and Medical Outcomes Study 36-item Short Form Survey (SF-36).

Adverse experiences are monitored throughout therapy and through follow-up. Baseline, one-week post, and six-month post therapy measurements are conducted. This design will elucidate the efficacy of EECP in functional performance and quality of life in patients with mild to moderate symptoms of heart disease over that of optimal usual care guideline-mandated medical therapy.

The results from the PEECH trial are not published regarding the beneficial effects of EECP in patients with a LVEF less than 35%. However, two papers investigate possible mechanisms of actions that can be attributed to the beneficial effects seen due EECP therapy, specifically involving left ventricular measures. A 2001 Japanese study (70) investigated EECP endpoints pertaining to exercise tolerance, ischemia, and left ventricular diastolic filling in patients with CAD. After excluding patients based on contraindications, twelve patients (10 men) were enrolled in the study. All patients had residual vessel disease, most with previous MI, PTCA, and/or CABG (only three had none), a multitude of coronary risk factors (one with none), and extensive medication usage (all had three of more prescriptions). The study involved two different phases. The first involved a control period (average of 38 days) involving sedentary or mild activity and no EECP therapy. Medical histories, examinations, and exercise stress tests (standard Bruce) were performed as a baseline evaluation. Exercise duration, exercise tolerance, time to 1-mm ST-segment depression, rate-pressure product (RPP) at peak exercise, and RPP at 1-mm ST-segment depression were measured. The second phase was 35 hours of EECP treatment, performed on

an in-patient basis to better control treatment. Treatment was given once or twice a day and lasted 36 ± 6 days. Before and after EECP treatment, exercise stress tests, exercise thallium-201 scintigraphy, and cardiac catheterization (including left ventricular and selected coronary angiography) were performed. Venous blood samples were obtained before and after EECP treatment to determine blood concentrations of atrial (ANP) and brain natriuretic peptides (BNP).

EECP treatment improved exercise duration (seconds), exercise tolerance (METS), time to 1-mm ST-segment depression, RPP at peak exercise, and RPP at 1-mm ST-segment depression. Before EECP half of 156 segments in the 12 subjects were identified via thallium-201 imaging as normal, the other half identified as abnormal. After therapy, 104 (67%) were classified as normal perfusion, a significant increase in myocardial perfusion. The hemodynamic and collateral vessel responses to EECP changed significantly in only the left ventricular end-diastolic pressures. Although resting heart rate did not change, there was an average decrease from 66 to 63 bpm. Additionally, peak filling rate significantly increased and time to peak filling rate significantly decreased after treatment. Interestingly, LVEF did not change. Biological markers associated with left ventricular stress and/or stretch also changed due to EECP. BNP decreased following EECP treatment, while ANP showed an average, although non-significant, decline following treatment. Contact dermatitis was the only adverse event cited. Thus, the positive adaptations due to EECP were related

specifically to exercise tolerance, myocardial perfusion, and left ventricular diastolic filling.

Effects on acute and chronic hemodynamics

Michaels, et al. (45) assessed intracoronary, central aortic, and cardiac hemodynamics during EECP to determine whether these acute effects translate into a favorable outcome for patients with disorders such as acute coronary syndrome or cardiogenic shock. Ten outpatients referred for cardiac catheterization and coronary angiography were enrolled in this study to evaluate acute responses to a single session of EECP therapy. The EECP treatment resulted in a decrease in average peak aortic systolic pressure (pre = 114 mmHg, post = 101 mmHg, $p = 0.02$), an increase in average aortic diastolic pressure (pre = 71 mmHg, post = 136 mmHg, $p < 0.0001$), and an increase in mean aortic pressure (pre = 88mmHg, post = 102 mmHg, $p = 0.0007$). There was also a trend for a decrease in left ventricular end-diastolic pressure (pre = 15 mmHg, post = 13 mmHg, $p = 0.17$). Significant increases were observed for intracoronary peak diastolic pressure (pre = 71 mmHg, post = 137 mmHg, $p < 0.0001$), and mean pressure (pre = 88 mmHg, post = 102 mmHg, $p = 0.006$), with a concomitant decrease in peak systolic pressure (pre = 116 mmHg, post = 99 mmHg, $p = 0.002$). These data suggests that EECP therapy decreases workload on the heart, at least during EECP treatment, due to a decrease in resistance attributable to the counterpulsation.

There are two major notes of interest concerning this study: 1) Some patients received pressures as low as 100 mmHg, suggesting that those receiving doses of 280-300 mmHg could possibly see greater responses; 2) The application of EECP was performed once, suggesting that patients receiving the full course of a 35-hour prescription may experience even better responses. In support of this hypothesis, a 2001 study (72) determined the effects of EECP on ocular blood flow velocities, comparing healthy volunteers and patients with atherosclerosis. Flow velocities of the ophthalmic artery were measured by Doppler sonography in 12 healthy volunteers (mean age 31.3 $\pm$ 4.3, 9 male) and 12 patients (mean age 62.1 $\pm$ 5.3, 8 male) with severe atherosclerosis. At baseline, the atherosclerotic patients exhibited lower, albeit nonsignificant, values for mean flow velocity, mean systolic flow velocity, and mean diastolic flow velocity compared to the healthy controls. Following the acute EECP treatment session, the diastolic flow velocity increased nonsignificantly from 21 to 23 cm/s and systolic flow velocity decreased from 36 to 29 cm/s ($p < 0.01$). These responses in the healthy group resulted in no overall significant change of mean flow velocity (pre = 28 cm/s, post 26 cm/s). Atherosclerotic patients exhibited an increase in diastolic flow velocity from 20 to 24 cm/s ($p < 0.001$), a nonsignificant reduction in systolic flow velocity from 34 to 33 cm/s, and an overall significant increase in mean flow velocity from 26 to 29 cm/s ($p < 0.01$). These findings suggest that change in mean flow velocity could ultimately depend on the status of the patient relative to the presence of CVD.

Dockery, et al. (16) recently explored the impact of EECP on arterial wall stiffness to determine if stiffness is augmented during EECP. Patients included those referred with refractory angina, CCS class of II-IV, who were considered unsuitable for revascularization. Patients received 35 hours of EECP treatment at one-hour intervals daily, with cuff pressures at 300 mmHg. Arterial stiffness was evaluated using pulse wave velocity and determined before and after treatment. Seventeen patients underwent baseline and post-EECP therapy exercise treadmill tests, using the standard Bruce protocol. Twenty-three of 25 patients completed therapy, with 19 available for the six-month follow-up.

Brachial systolic and diastolic blood pressures decreased significantly from baseline to post-EECP (138/79 to 132/73 mmHg, respectively, $p < 0.05$). Heart rate showed a nonsignificant decrease from 63 to 61 beats per minute, pre to post-EECP, respectively ($p = 0.25$). Markers of arterial stiffness did not change from pre to post-EECP. Furthermore, exercise duration did not improve significantly. These findings suggest that the benefits seen with EECP are not attributable to changes in arterial stiffness, at least when measured by pulse wave velocity or aortic augmentation index, possibly discrediting the nature of the vasculature theory proposed by Werner, et al. (72). However, it is still possible that there are changes in the nature of the vasculature (i.e. enhanced ability to increase vasodilators, decrease vasoconstrictors, etc.) but the flow is ultimately controlled by autoregulation.

Effects on angiogenesis

Previous studies demonstrate that EECP improvements are due to angiogenesis rather than altered myocardial oxygen demand. A recent multicenter trial (64) evaluated the efficacy of EECP in improving exertional ischemia in patients with chronic stable angina using radionucleotide perfusion treadmill tests. A baseline maximal treadmill test was performed using a Bruce protocol and either technetium-99m or thallium-201 and single-photon emission computed tomography or planar imaging. Patients underwent EECP, using daily, one-hour sessions with the therapeutic range of pressure between 225 to 275 mmHg. Following 35 hours of therapy, the same imaging technique was used in a post-EECP treadmill test. Four centers conducted the post-EECP treadmill test to the same level of exercise as the patients' pre-EECP treadmill test; the other three centers conducted post-EECP maximal treadmill tests.

Two different protocols for post-EECP tests allowed for analysis of both changes in the maximal and submaximal exercise responses following EECP therapy. Improvements in CCS class were reported in 85% of patients, with 15% reporting improvements in two or more classes. In the centers where patients were post-tested to the same level of exercise, 83% had significant improvements in perfusion defects, 17 percent showed no improvements and none worsened. Patients who conducted post-EECP maximal treadmill tests increased exercise duration (6.61 ± 1.88 vs. 7.41 ± 2.03 minutes pre to post, $p < 0.0001$), with no change in double product. This supports the hypothesis that angiogenesis occurred due to increased blood supply without an alteration in

maximal myocardial oxygen demand. The majority of these patients (54%) showed improvements in perfusion defects, 42% had unchanged perfusion defects, and four percent regressed. Additionally, patients who did not perform a post-EECT maximal treadmill test, but rather performed at the same exercise level as their pre-EECP maximal treadmill tests, experienced a lower double product on their post-EECP treadmill tests. This reflects a decrease in the myocardial oxygen demand at a given submaximal workload. These results may be even more profound given that post-EECP treadmill tests were conducted within six months of the last EECP treatment. The prolonged time in capturing the post-EECP test could have missed an even larger change that may have, at least in part, dissipated during the time between ending EECP therapy and having the post-EECP measurement taken.

It has also been proposed that the mechanism of action for improved perfusion due to EECP is the development of collateral channels and/or enhanced collateral flow. To test this hypothesis, Tartaglia, et al. (66) evaluated 25 patients (23 males, age 68±9) for perfusion defects before and after EECP therapy. At least one CCS class reduction was reported in 84% of patients, 12% reported a two CCS class reduction, and four percent (one patient) reported no improvement. Improved maximal treadmill times were reported in 94% of patients with 64% improving coronary perfusion. Thirteen of 16 patients exhibiting ST-segment depression on their pre-EECP treadmill test reported delays (n=10) or absence (n=3) of the depression on their post-EECP tests. Peak double product increased ($p < 0.03$), while peak blood pressures remained

unchanged. These data showed improved treadmill times and a reduction in reversible defects on the post-EECP treadmill tests and perfusion imaging. Increased treadmill times and perfusion are indicative of increased collateral flow. Unfounded speculation has suggested that peripheral muscular training effects increase exercise duration following EECP therapy. These peripheral effects would mean that there would be no change in, or even a decrease in, myocardial oxygen demand. These data do not support peripheral training as the sole source of benefits as shown by the increase in double product, indicating an increase in myocardial oxygen demand and flow.

To further evaluate whether EECP stimulates angiogenesis, Masuda, et al. (41) compared standard EECP therapy to EECP with additional heparin, which is known to potentiate the action of angiogenesis. The study sample included 18 patients between the ages of 40-80 years (13 men, age 62 $\pm$ 9 years). All had at least 70% stenosis involving one or more major coronary arteries and ST-segment depression during treadmill exercise testing. Eleven patients underwent traditional EECP, consisting of 35 hours of one-hour sessions, while seven patients were treated with EECP therapy and 5000 IU injections of heparin, given intravenously ten minutes prior to each EECP session. The heparin group only underwent 24 hours of treatment. Maximal exercise treadmill tests were performed prior to and upon completion of EECP treatment and myocardial perfusion (designated as either ischemia or nonischemic) was determined by acetate PET. Additionally, PET was utilized to calculate clearance rate constant, k mono, which is characteristic of regional

myocardial oxygen metabolism. Sublingual nitroglycerin use decreased significantly in both groups as a result of EECP. The heparin group increased maximal treadmill time from 353 ± 127 to 505 ± 168 seconds ($p < 0.01$), with the conventional group showing a nonsignificant increase from 390 ± 131 to 439 ± 135 seconds. The heparin group also experienced increased times to the onset of 1-mm ST-segment depression ($p<0.01$) and increased peak double product ($p<0.05$) compared to a nonsignificant increase in time to 1-mm segment depression and nonsignificant decrease in peak double product in the conventional group. Following EECP, the heparin group illustrated a significant increase in k mono in the designated ischemic areas and no change in the nonischemic areas. Therefore, despite fewer sessions of EECP, the heparin group had greater improvements in exercise tolerance, perfusion, and myocardial oxygen metabolism. Results suggest that angiogenesis may play a larger role in the benefits seen with EECP than improvements in collateral conductance.

This same group published a 2001 study (42) on conventional EECP to evaluate the role of nitric oxide and neurohumoral factors on EECP adaptations. Eleven patients underwent a treadmill test (standard Bruce protocol), ^{13}N-ammonia PET, and blood collection prior to and within four weeks of EECP. Resting myocardial perfusion increased 23% following EECP, improving in both CAD and non-CAD regions. Exercise tolerance tests revealed a delay in the onset of 1-mm ST-segment depression from 190.5 ± 115.5 seconds, pre-EECP to 375.5 ± 202.4 seconds post-EECP. Peak double product remained unchanged following EECP. One month following EECP, NO was increased

nearly two-fold ($p < 0.02$) compared to baseline values. After one day post-EECP ANP and BNP were significantly decreased. One-week post-EECP, values for ANP and BNP were still lower than baseline values ($p < 0.02$), but returned to nonsignificant levels within one month following post-EECP. The perfusion and NO data suggest improved coronary flow. Increased vasodilation due to NO would decrease vascular resistance and decrease workload of the left ventricle. Changes in A/BNP levels provide additional data to support this decreased workload of the left ventricle, as higher levels are indicators of left ventricular stress.

Effects on peripheral perfusion

Studies discussed in this literature review demonstrate the effectiveness of EECP on improving perfusion in the coronary vessels. Furthermore, recent data (23) suggest that EECP improves skin oxygenation and perfusion measured in the forehead and index finger. Prior research (21) also indicates that EECP is an effective treatment for erectile dysfunction (ED). The reported improvements could have been attributed to improved peripheral blood flow, or improved psychosocial parameters, or both. However, sonographic analysis also demonstrated improved peak systolic blood flow, which could have resulted in decreased ED. In a smaller sample of patients, cavernous blood was drawn (from the penile corpora cavernosa) before and after 20 hours of EECP therapy to analyze growth factors. Results of the blood draw did not show significant adaptations to growth factor levels due to EECP. The authors suggest

psychological improvements, specifically quality of life, as an explanation of the improvements seen in the patients, but collected no data to evaluate the hypothesis.

Fricchione, et al. (20) demonstrated that EECP therapy improved sexual activity, social life, and family life. Other improvements include health condition, overall well-being, ability to work, and energy level. A larger follow-up study (63) was published in 2001 by the same group of authors. This newer study aimed to analyze the psychosocial effects of EECP in greater depth and with a larger sample size than the previous study. Male subjects with angina and CAD were enrolled into the study and underwent 35 hours of EECP, pre and post-EECP stress radionucleotide scan, an exercise tolerance test, and a battery of questionnaires. Patients reported significantly fewer angina episodes, lessened angina severity, and decreased use of antianginal medications following EECP therapy. Furthermore, subjects with the largest improvements in perfusion and/or decreased symptoms of angina also had the largest improvements in general psychological distress, depression, anxiety, and somatization. As a group, 87% of patients reported some improvement in overall well-being, health conditions, energy level, and ability to work, regardless of improvement in ischemia. The authors note the possibility of a placebo effect, where the introduction into an environment of socialization and daily contact with a larger group of individuals with similar medical problems could impact the outcomes of the study. Another concern with this therapy is the potential strain on the quality of life of the patient if disruptions in family expectations and routines occur due to

the need for daily therapy. However, EECP has typically demonstrated improvements in quality of life.

Despite the physiological and psychological improvements seen with EECP, the mechanism of action remains unclear. Improved coronary perfusion has been suggested as the mechanism of action. Evidence attributes flow-mediated vasodilation as reason for increasing coronary perfusion. High amounts of sheer stress result in endothelial release of nitric oxide (NO), an important vasodilator, anti-platelet, anti-thrombotic, and anti-inflammatory properties. Conversely, low amounts of sheer stress are associated with endothelial release of endothelin-1, a potent vasoconstrictor. NO is known to inhibit the release of endothelin-1.

Effects of endothelium

Similar to Masuda, et al.(42) who reported an increase in NO following EECP, Wu, et al. (73) published an abstract reporting the effects of EECP in vasoactive substances (derived from endothelial cells), specifically NO and endothelin-1. Forty-three coronary heart disease patients underwent 36 hours of EECP treatment with plasma samples taken pre-EECP, after the 1^{st}, 12^{th}, 24^{th}, 36^{th} hours of EECP, and at one and three months post-EECP. Plasma NO exhibited an increase proportional to the amount of hours of treatment. Endothelin-1 showed a similar proportional, but decreased, response during treatment. One month following treatment, NO levels remained elevated with endothelin-1 levels remaining decreased, compared to baseline. Three-month

post-EECP data showed a continued trend of these changes, but they were no longer statistically significant. These data suggest that EECP results in a sustained increase in NO and a decrease in endothelin-1, both of which could improve flow-mediated vasodilation.

Shechter, et al. (57) and Bonetti, et al. (7) suggest that EECP improves endothelial-dependent vasoreactivity, which subsequently increases perfusion. Shechter, et al. (57) hypothesized that EECP therapy would increase flow-mediated dilation in CAD patients with angina. Twenty patients (15 males, age 68 ± 11 years) referred to EECP and an additional 20 (17 males, age 67 ± 12 years) age- and gender-matched controls, who elected not to participate in EECP therapy, were enrolled into the study. Patients were advised not to change diet and medication at the onset on therapy, which consisted of 35 hours of EECP for the patients and two months of no intervention for the controls. All 40 patients underwent a physical examination, brachial artery reactivity testing, CCS class assessment, and were asked to list the number of anginal episodes experienced and the number of nitroglycerin tablets taken.

Following EECP therapy, the patients' flow-mediated-dilation increased from 3% to 8% ($p = 0.01$), pre to post-EECP. The control group reported a two-month post of 3.1% (p = NS). Nitroglycerin-mediated vasodilation remained unchanged in both groups from pre to post, suggesting that the improved vasodilatory response was entirely due to adaptations in the endothelial cells. The EECP patient group reported a decrease in daily use of nitroglycerin from 4.2 to 0.4 nitroglycerin tablets per day ($p < 0.001$) with the control reporting no

significant difference between time points. The EECP patient group also reported decreases in CCS classification ($p < 0.0001$) with no significant changes in the control group.

Bonetti, et al. (7) investigated the effects of EECP on peripheral endothelial function in patients with advanced CAD. However, vascular reactivity was measured in the finger. All patients underwent the standard 35-hour course of EECP therapy, with treatments occurring at the same time of day. Patients underwent pre and post-EECP acute reactive hyperemia testing, with an additional follow-up measure taken one month after EECP. Additionally, on all study days, data on CCS classification, cardiovascular functional status, and functional capacity questionnaires were collected. Cardiovascular medication remained unchanged during treatment. Twenty-three patients (22 male, age 66 $\pm$ 2) received 35 hours of EECP therapy. Eighteen of those patients were measured at the one-month follow-up. Twelve patients improved CCS classification by one class, another five patients improved their classification by two classes, and ix patients reported no change in CCS classification. Seventeen patients reported improvements in their functional status, although there were no acute nor chronic effects of EECP on heart rate or blood pressure. Acute improvements in reactivity were seen on all of the days measured with the improvements continuing to show significance at the one-month measurement. These data show that EECP improves acute peripheral arterial reactivity, as measured in the index finger, and attributable to improvements in endothelial function. This finding further supports the suggestion that improved endothelial

function mediates the benefits experienced by patients undergoing EECP. Specifically, those who reported higher post-EECP activity scores and an improved CCS angina classification experienced greater improvements in endothelial function, as quantified by reactive hyperemic response.

When comparing patients with CCS classification improvements to those without CCS improvements, the post-EECP reactivity improved from the baseline measurement to the one-month follow-up in only the patients who improved their CCS classification. The same is true for those reporting improvements in their functional status. Improvements due to EECP, i.e. CCS class improvement and reported functional status, are associated with increased reactivity of peripheral vessels.

Coagulative and fibrinolytic considerations for EECP treatment

Few data exists documenting fibrinolytic or coagulative adaptations in response to acute treatments or chronic therapy of EECP. In fact, the most available evidence must be inferred from intermittent pneumatic compression (IPC) studies. IPC has proven to be an effective form of deep vein thrombosis (DVT) prevention for patients that have been immobilized following surgery.

A study (9) evaluating the mechanisms for enhanced fibrinolysis due to IPC was performed with five different IPC devices. Six healthy patients and six patients with a history of DVT were enrolled. Thigh length and calf length sequential compression, thigh length and calf length single-chamber compression, and a foot pump were the five devices used on all patients. They

received a different IPC for 120 minutes, once a week, for five weeks. Blood samples were obtained at baseline, 60 minutes into therapy, immediately post treatment (120 minutes), and one-hour post treatment (180 minutes). Overall fibrinolytic activity was measured using euglobulin fraction of plasma on a fibrin plate. Plasma tPA antigen and activity, PAI-1 antigen and activity, plasmin alpha-2-antiplasmin complex, and von Willebrand factor (vWF) were also measured.

There were no differences observed between the compression devices for any measurements taken. Results were then reported on an average of the five devices for each variable. Based on the analysis, fibrinolytic activity increased in both groups, increasing in the patients with a history of DVT to a post-treatment level similar to the baseline measurement of the healthy subjects. In patients with a history of DVT, tPA antigen decreased seventeen percent ($p = 0.001$), tPA activity remained unchanged, PAI-1 antigen decreased twelve percent ($p = 0.013$), and PAI-1 activity decreased seventeen percent ($p = 0.004$). Of interest, vWF remained unchanged and plasmin alpha-2-antiplasmin complex values decreased ten percent ($p = 0.021$).

tPA antigen decreased acutely, likely due to increased flow rate and a subsequently enhanced liver clearance. Overall improvements in patients with a history of DVT were greater than healthy controls and the authors contribute this to these patients' endothelial dysfunction. Considering the pressures exerted during a treatment of EECP, similar findings should be expected. Other differences between IPC and EECP is the length of time (EECP = 60 minute

sessions vs. IPC = 120 minute sessions), total hours of therapy (EECP = 60 minutes per session, five session per week, for seven weeks = 2100 hours of therapy), and intensity of therapy (EECP = 260 – 300 mmHg vs. IPC = 40 mmHg).

Chouhan, et al. published an additional article (8) in 1999 to examine a possible antithrombotic effect of acute IPC treatment. Although not stated directly, it appears the same data/plasma was used in this study as was used in their previous report (9). Additionally, much of the methodology was the same. Factor VIIa, FVII antigen, tissue factor pathway inhibitor (TFPI) antigen, prothrombin fragment F1.2, and antithrombin activity were measured in response to the five different devices. There were no differences between groups in the baseline values of variables measured. FVIIa levels decreased at each time point (60, 120, 180 minutes) in both groups ($p < 0.001$). There were no significant changes in FVII antigen in either group due to IPC treatment. TFPI increased significantly in both patient groups ($p < 0.001$), although the healthy controls experienced a higher response during IPC than the patients that had a history of DVT. There were no significant changes due to IPC with respect to both F1.2 and plasma antithrombin activity. A major finding of the study was that IPC increases tissue factor pathway inhibition, as measured by plasma TFPI levels, which is the principal regulator of the tissue factor pathway of blood coagulation. An additional major finding is the associated decrease in plasma FVIIa.

The only study involving the response of hemostatic factors and EECP therapy was published in 2005 by Arora, et al. (4). Specifically, the investigators examined the effects of EECP on the endothelial function in patients with CAD who have angina. Thirty patients were enrolled in the study and underwent the traditional 35 hours of EECP therapy. Blood was obtained prior to and following EECP therapy from a peripheral vein. There was no mention of time of day for the blood draw or fasting status of the patients. Plasma levels of tPA antigen, PAI-1 antigen, vWF, fibrin D-dimer, and vascular endothelial growth factor (VEGF) were measured. EECP did not elicit any changes in hemostatic factors or VEGF levels. The authors noted a nonsignificant increase in VEGF, possibly indicating angiogenesis. Acute responses pertaining to these variables were not measured as a result of one session of EECP; neither were tPA activity nor PAI-1 activity.

Mechanistically, EECP may affect fibrinolysis by simply improving the function of endothelial cells that are presently a part of the vasculature. Additionally, improved perfusion and/or angiogenesis may actually increase the absolute number of healthy, active endothelial cells that are able to perform normative activities, i.e. fibrinolytic responsibilities. These endothelial adaptations could be the result of coronary tissues specifically, or additionally in the systemic vasculature. Improved endothelial function would have a positive effect on tPA activity and antigen. Additionally, healthier endothelial tissue could potentially result in less activation of the TF/coagulation pathway. The subsequent decrease in coagulation would result in less platelet aggregation,

possibly affecting PAI-1. Lastly, EECP, in improving exercise tolerance, episodes of angina, quality of life, et cetera, may increase patients' abilities and desires to be physically active, thus improving their hemostatic profile.

Therefore, the specific aims of the current study are to determine: 1) the chronic effects of 35 sessions of EECP on fibrinolytic markers, specifically tPA activity, tPA antigen, PAI-1 activity, and D-dimer, and on a global marker of coagulation, TAT; and 2) the acute response of one 60-minute session of EECP on the fibrinolytic variables tPA activity, tPA antigen, and PAI-1 activity.

Chapter Three

Methods

Subjects

Twelve patients were enrolled into the study. Descriptive characteristics of these patients are displayed in Table 1. Patients were recruited from all patients referred for EECP treatment at William Beaumont Hospital's Cardiac Rehabilitation Center. Those who agreed to participate were given informed consent and standard EECP therapy. A 2003 review article in *Heart* (58) lists the contraindications and the reasoning for each as follows: within two weeks after cardiac catheterization or arterial puncture (risk of bleeding at femoral puncture site), arrhythmias that may interfere with triggering of EECP system (atrial fibrillation, flutter, and very frequent premature ventricular contractions), decompensated heart failure, usually class III to IV (EECP results in an increase in venous return), left ventricular ejection fraction <30% (increased preload may precipitate heart failure), moderate to severe aortic insufficiency (regurgitation would prevent diastolic augmentation), severe peripheral arterial disease (reduced vascular volume and muscle mass may prevent effective counterpulsation, increased risk of thromboembolism), severe hypertension > 180/110 mmHg (the augmented diastolic pressure may exceed safe limits), aortic aneurysm or dissection (diastolic pressure augmentation may be deleterious), pregnancy or women of childbearing age (effect of EECP on fetus have not been studied), venous disease (phlebitis, varicose veins, stasis ulcers, prior or current

deep vein thrombosis or pulmonary embolism), severe chronic obstructive pulmonary disease (no safety data in pulmonary hypertension), and coagulopathy with international normalized ratio of prothrombin time > 2.0 (to avoid risk of haemotoma with high cuff pressures).

Therapy

Participants underwent traditional EECP treatment (60 minutes/session, 5 sessions/week for a total of 35 sessions). Body weight and blood pressure were taken prior to EECP treatment. Each treatment session involved patients lying supine with three sets of cuffs wrapped around the calves and the lower and upper thighs. Patient EKG controlled inflation and deflation of the cuffs. The cuffs inflated sequentially (calf, lower then upper thigh) at the onset of diastole and rapidly released pressure prior to systole. Therapeutic pressure of 260-280 mmHg was obtained by the end of the fifth session.

Blood sampling

For chronic analysis, blood samples were drawn within a week prior to the start of treatment and within a week following the last treatment date. For acute analysis, blood was drawn immediately prior to and within three minutes of completing a treatment session. Chronic blood draws were performed between 7:00 and 10:00 o'clock in the morning to minimize diurnal variation (3). Chronic samples were taken following 15 minutes in a seated position. Acute samples were obtained with the subject in a supine position on the EECP treatment table

during their regularly scheduled appointment throughout the day. The acute blood draws were taken following at least 15 minutes of rest with the exception of the acute post treatment draw, which was obtained within 3 minutes of the cessation of treatment. The venous blood samples were obtained from an antecubital vein with 5 ml of blood was drawn in to an EDTA tube then discarded and an additional 5 ml of blood was drawn into a chilled acidified citrate (Stabilyte tube, Biopool, Int.) solution. Platelet-poor plasma was obtained from these samples and stored at -80°C until assayed.

Blood assays

tPA and PAI-1 activity were determined using a bio-functional immunosorbent assay (BIA) (Biopool, Int. Sweden). tPA antigen was determined using enzyme-linked immunosorbancy assays (ELISA) (American Diagnostica inc. Greenwich, CT). D-dimer was measured using an ELISA (American Diagnostica inc. Greenwich, CT) to quantify the amount of crosslinked fibrin degredation products in human plasma. Thrombin Antithrombin Complex (TAT) was used as a marker of coagulation and was determined using ELISA (Dade Behring Marburg. Newark, DE). All samples were run in duplicate on the same plate, with lab inter- and intra-assay variations under five percent.

Statistical analyses

Acute pre and post-EECP treatment session samples of tPA activity, tPA antigen, and PAI-1 activity were analyzed using tailed, dependent t-tests.

Baseline and post-35 sessions of EECP samples of tPA activity, tPA antigen, PAI-1 activity, D-dimer, and TAT were analyzed using tailed, dependent t-tests. Directionality of the t-test was set by the determination of the hypotheses. Data was also analyzed for abnormal distributions and transformed. PAI-1 activity and d-dimer data were abnormally distributed and log transformed for analysis. Significance level for all tests was set at $p \leq 0.05$.

Chapter Four

Results

Ten of twelve patients had complete data for the acute bout of EECP treatment. Blood samples could not be obtained on the other two patients. On average, data were collected for the acute bout during session 16 with a range of 8 - 28 sessions (at the end of the second week up to the end of the sixth week). Eleven of twelve subjects had complete data for the chronic EECP therapy. One subject did not complete the 35 sessions, due to reported back pain. Hemodynamic adaptations to the EECP therapy are displayed in Table 1. There was a significant decrease in resting systolic blood pressure over the course of therapy (pre = 125.8 ± 21.2 mmHg, post = 116.4 ± 17.8 mmHg, p = 0.012). Patients also exhibited nonsignificant changes in resting diastolic blood pressure (pre = 69.6 ± 10.0 mmHg, post = 65.5 ± 8.0 mmHg, p = 0.097) and weight (pre = 87.6 ± 16.1 kg, post = 86.5 ± 16.2 kg, p = 0.14), although resting heart rate remained unchanged (pre = 66.0 ± 9.2 bpm, post = 66.8 ± 8.4 bpm, p = 0.82).

Acute responses to EECP treatment

Figure 1 displays plasma tPA activity, tPA antigen, and PAI-1 activity in response to acute EECP treatment, with the group average in bold. With eight of the ten patients showing an increase in tPA activity, tPA activity increased significantly (pre = 0.80 ± 0.57 IU/ml, post = 1.00 ± 0.57 IU/ml, p = 0.01) due to one bout of EECP. However, there were no significant changes in plasma tPA

antigen (pre = 10.18 ± 2.38 ng/ml, post = 9.99 ± 2.20 ng/ml). Additionally, there was a significant decrease in plasma PAI-1 activity after an acute treatment session (pre = 26.48 ± 42.63 IU/ml, post = 19.65 ± 33.15 IU/ml, p = 0.027). Eight out of ten patients experienced a decrease in PAI-1 activity.

Chronic adaptations to EECP therapy

Figure 2 displays the chronic adaptations to EECP therapy. There was a nonsignificant trend (pre = 0.79 ± 0.46 IU/ml, post = 0.65 ± 0.40 IU/ml, p = 0.061) towards a decrease in plasma tPA activity. There were no significant changes in plasma tPA antigen (pre = 10.40 ± 2.67, post = 10.38 ± 2.60 or plasma PAI-1 activity due to the EECP therapy (pre = 19.15 ± 20.41 ng/ml, post = 20.11 ± 18.11 ng/ml, p = 0.85). Additionally, d-dimer did not change as a result of the therapy (pre = 574.90 ± 535.73 ng/ml, post = 462.78 ± 280.23, p = 0.30). There was a nonsignificant increase in plasma TAT (pre = 1.71 ± 0.60 ug/L, post = 2.49 ± 1.49 ug/L, p = 0.08), with seven of the eleven patients showing an increase.

Chapter Five

Discussion

The major clinical finding of the present study is that acute bouts of EECP enhance fibrinolytic potential by increasing tPA activity and decreasing PAI-1 activity. Because patients receive 35 hours of therapy over the course of seven to eight weeks, it is possible that these acute, transient improvements are clinically significant in those with impaired fibrinolysis. These acute data are similar to previous findings (9) where the authors noted an acute increase in tPA activity and decrease in PAI-1 activity in response to one session of intermittent pneumatic compression (IPC) therapy in patients with a history of deep vein thrombosis. The present findings confirm these previous data and suggest that EECP therapy also elicits similar beneficial acute fibrinolytic responses in patients with CAD and resultant angina. The acute improvements demonstrate daily improvements in fibrinolysis.

The chronic adaptation is indicative of the increased inhibition of the coagulation pathway. Although not statistically significant, most patients experienced a decrease in d-dimer and an increase in TAT. Antithrombin is a plasma-derived inhibitor that prevents the further development of coagulation by inhibiting certain coagulation factors (53). Antithrombin binds thrombin to form the inactive TAT complex also inhibits other processes in the coagulation cascade, such as activated Factor XI, activated Factor IX, and activated Factor X. While inhibition of the activated forms of Factors XI and IX are in the contact

factor pathway, activated Factor X and thrombin are in the common pathway. Inhibition of either of these common pathway markers would result in the retarded formation of fibrin clots. This could have profound effects on the patients improved ability to retard hemostasis and coagulation. The inhibition of the coagulation pathway at multiple points, although TAT was the only marker measured in the present study, would decrease the formation of fibrin clots. This inhibition of the coagulation pathway is in agreement with a study by Chouhan, et al. (8) demonstrating that the TF pathway was inhibited by TFPI, thus slowing the tissue factor pathway and ultimate fibrin production in response to IPC therapy.

The nonsignificant decrease in d-dimer is also interesting when taking into account the trend for increased TAT. Because plasma d-dimer concentration represents the end products of fibrinolysis, decreased thrombogenesis, along with a decreased, or unchanged, fibrin degradation products (d-dimer) would suggest systemic fibrinolysis. Essentially, the data show a decrease in clot formation, which possibly led to a decreased amount of clot lysis products.

Our data support the findings of the only other study (4) that evaluated the effects of EECP on hemostasis that showed von Willebrand Factor, tPA antigen and d-dimer levels to be unchanged following a course of 35 hours of EECP therapy. The authors, however, did not measure any acute outcomes, chronic fibrinolytic activities, or coagulation markers in their study.

There were significant reductions in systolic blood pressure and trends towards decreases in diastolic blood pressure and weight loss observed with chronic EECP therapy. Collectively, these benefits could positively impact

lifestyle choices where continued improvements can further enhance improved hemostasis and fibrinolysis. While this study does not elucidate the mechanism by which these benefits are realized, these data do support the hypothesis of shear stress as a modulator. Diastolic augmentation has been demonstrated (46) to affect shear stress, which impacts NO production. Increased NO is a demonstrated result of EECP (42), suggesting an improved endothelial function and vasoreactivity. Alternatively, increased endothelial function would likely have a positive affect on tPA synthesis and release, and subsequently plasma tPA activity and antigen. Future studies should evaluate the mechanistic causes of hemodynamic adaptations to EECP therapy.

The limitations of the present study are possible placebo effects from the social interactions of intervention. The decreased systolic blood pressure could be a product of nervousness at the onset of therapy compared to a relaxed, comfortable environment at the conclusion of therapy. External validity may not be highly robust, considering the socioeconomic status of the group of patients in the Royal Oak, and surrounding areas. Specifically, patient retention could be positively affected in this group. Improved screening and enhanced previous medical attention could also be a factor.

In summary, the present data suggest beneficial acute fibrinolytic responses to a single session of EECP. Chronically, nonsignificant trends suggest improved inhibition of the coagulation pathway, potentially negating the necessity of improved chronic fibrinolytic capacities. It is possible that transient improvements in fibrinolysis resulting form EECP therapy could provide

temporary hemodynamic improvements that decrease ischemic risk. Furthermore, these data confirm positive hemodynamic adaptations to EECP therapy in CAD patients as exhibited by significant decreases in systolic blood pressure.

APPENDIX A

Tables

Table 1. Patient characteristics

	(N = 12)		p value
	Pre	post	
Age (years)	69.3 ± 7.9		
Gender (% males)	66.7		
Height (cm)	172.6 ± 8.8		
Weight (km)	87.6 ± 16.1	86.5 ± 16.2	0.14
Heart Rate (bpm)	66.0 ± 9.2	66.8 ± 8.4	0.82
SBP (mmHg)*	125.8 ± 21.2	116.4 ± 17.8	0.01
DBP (mmHg)	69.6 ± 10.0	65.5 ± 8.0	0.10

Data displayed as mean ± standard deviation. *bpm* = beats per minute, *SBP* = systolic blood pressure, *DBP* = diastolic blood pressure
p value is reflective of chronic pre values compared to chronic post values.
*-Post-therapy value significantly different than pre-therapy value ($P < 0.05$)

APPENDIX B

Figures

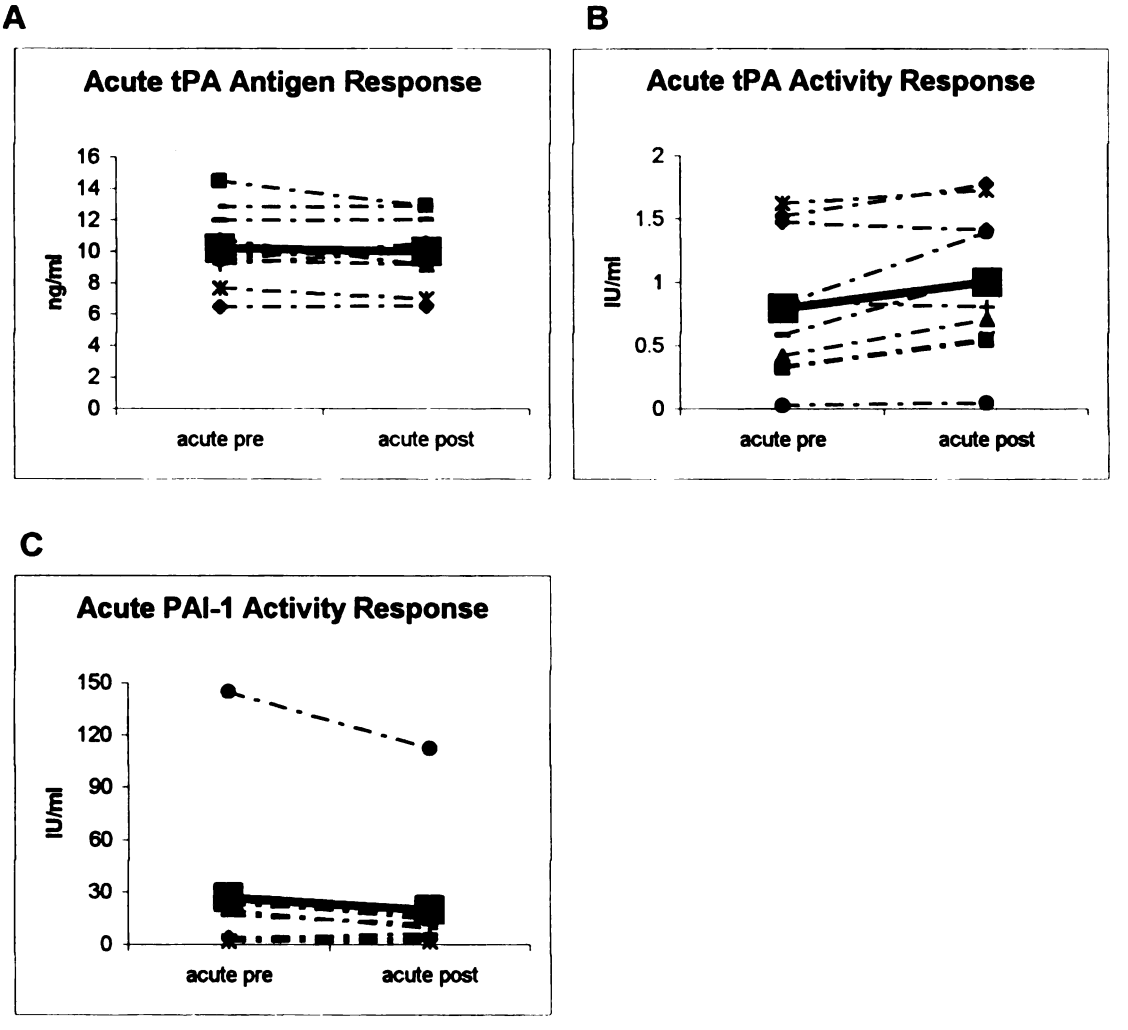

Figure 1. Acute responses to EECP treatment
Acute responses for individual (dashed lines) and group mean (heavy line) for (A) tPA activity ($p = 0.012$), (B) tPA antigen ($p = 0.523$), and (C) PAI-1 activity ($p = 0.027$).

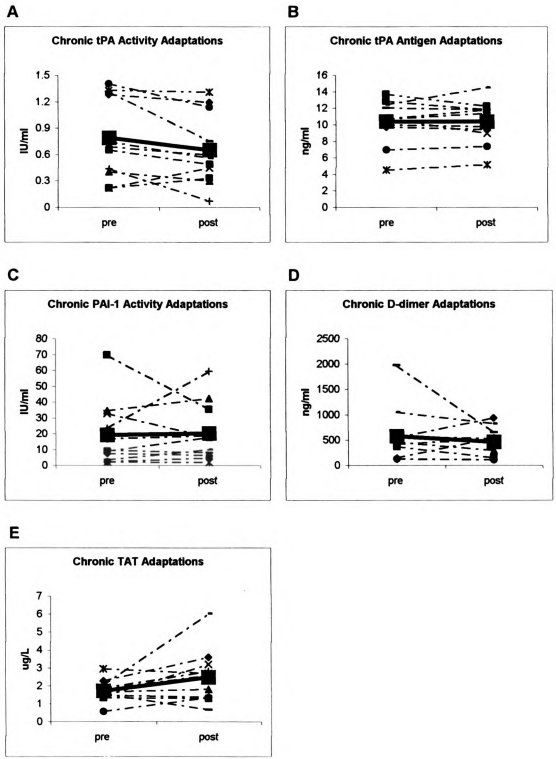

Figure 2. Chronic adaptations to EECP therapy
Chronic adaptations for individuals (dashed lines) and group mean (heavy line) for (A) tPA activity ($p = 0.061$), (B) tPA antigen ($p = 0.946$), (C) PAI-1 activity ($p = 0.855$), (D) d-dimer ($p = 0.30$), and (E) TAT ($p = 0.08$).

APPENDIX C

UCRIHS Approval

MICHIGAN STATE
UNIVERSITY

April 13, 2004

TO: Christopher WOMACK
3 IM Sports Circle
MSU

RE: **IRB# 04-172** CATEGORY: FULL REVIEW

APPROVAL DATE: April 5, 2004
EXPIRATION DATE March 5, 2005

TITLE: ACUTE AND CHRONIC EFFECTS OF ENHANCED EXTERNAL COUNTERPULSATION THERAPY IN PATIENTS WITH CARDIOVASCULAR DISEASE

The University Committee on Research Involving Human Subjects' (UCRIHS) review of this project is complete and I am pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and methods to obtain informed consent are appropriate. Therefore, the **UCRIHS approved this project.**

RENEWALS: UCRIHS approval is valid until the expiration date listed above. Projects continuing beyond this date must be renewed with the renewal form. A maximum of four such expedited renewals are possible. Investigators wishing to continue a project beyond that time need to submit a 5-year application for a complete review.
REVISIONS: UCRIHS must review any changes in procedures involving human subjects, prior to initiation of the change. If this is done at the time of renewal, please include a revision form with the renewal. To revise an approved protocol at any other time during the year, send your written request with an attached revision cover sheet to the UCRIHS Chair, requesting revised approval and referencing the project's IRB# and title. Include in your request a description of the change and any revised instruments, consent forms or advertisements that are applicable.
PROBLEMS/CHANGES: Should either of the following arise during the course of the work, notify UCRIHS promptly: 1) problems (unexpected side effects, complaints, etc.) involving human subjects or 2) changes in the research environment or new information indicating greater risk to the human subjects than existed when the protocol was previously reviewed and approved.

If we can be of further assistance, please contact us at (517) 355-2180 or via email: UCRIHS@msu.edu. Please note that all UCRIHS forms are located on the web: http://www.humanresearch.msu.edu

Sincerely,

Peter Vasilenko, Ph.D.
UCRIHS Chair
PV: kj

cc: Adam Coughlin
12024 Juniper Way Grand
Blanc, MI 48439

APPENDIX D

Consent Form

ACUTE AND CHRONIC EFFECTS OF ENHANCED EXTERNAL COUNTERPULSATION THERAPY IN PATIENTS WITH CARDIOVASCULAR DISEASE

- **INTRODUCTION**

You are invited to participate in a research study to determine the effects of Enhanced External Counterpulsation (EECP) on blood clotting factors, blood flow in the extremities (legs and arms), blood sugar values and activities of daily living. If you are eligible and decide to participate in the study, you will be one of 20 patients included in this research study.

- **DESCRIPTION OF THE STUDY**

The EECP therapy used in this study is permitted by the United States Food and Drug Administration (FDA) to treat patients with chronic chest pain. The only experimental aspect of this study is the determination of the effect EECP has on blood clotting factors, blood flow in the extremities, blood sugar values and activities of daily living.

- **ALTERNATIVE OPTIONS:**

You do not have to participate in this study to receive treatment for your condition. One option may be to refuse enrollment in this study and receive the standard of care which is EECP therapy.

- **STUDY PROCEDURES:**

This study consists of four elements: 1) Doppler Study; 2) Blood Draw; 3) Pedometer Study; and 4) Treatment with EECP.

1) Doppler Study

This procedure is a non-invasive study using ultrasound that determines blood flow in the legs and arms. These studies will be conducted within one week prior to beginning EECP therapy and within one week after completing EECP therapy.

2) Blood Draw

The blood samples will be analyzed to determine various blood clotting factors. There will be a total of four blood draws performed during this study. Two of the blood draws will be completed prior to your initial treatment and within one week of completing your final treatment at the location of your EECP therapy. Blood samples will be obtained from an arm vein. For these two blood draws, you will need to be fasted (no food or drink except water) for 12 hours prior to the blood draw, which will take place in the morning, before 10AM.

The other two blood draws will be performed within your first two weeks of EECP therapy. These samples will be obtained immediately prior to and immediately following one session of EECP therapy to determine the effect that one session of EECP has on blood coagulation. You do not need to be in a fasting state for these blood draws.

For each blood draw, we will obtain 25 cc of blood, which is approximately 2 tablespoons. Each blood draw will take approximately 20 minutes.

3) Pedometer
You will be asked to wear a pedometer for two 48-hour periods within one week prior to and after completing the EECP therapy schedule. These two 48-hour periods must fall on the same two days of the week. You will be asked to assume their normal daily activities during this phase of the evaluation.

4) EECP Therapy
EECP is a treatment used to treat chronic chest pain. Treatment consists of a series of blood pressure-like cuffs that are wrapped around the calves, thighs and buttocks. The blood pressure-like cuffs inflate with air between heartbeats and deflate while the heart is pumping blood to the body. This pressure applied to the legs forces blood back to the heart, which increases the amount of blood returning to the heart. EECP therapy may increase the amount of blood reaching the heart's tissue, in turn decreasing the number of episodes and severity of chest pain.

The total amount of time required for this study is approximately one week prior to beginning and one week after completing EECP therapy schedule.

Your participation in this study will provide valuable information to doctors and EECP therapists on the associated effects of this relatively new treatment.

The following information is to inform you of potential risks/benefits so you can decide with confidence whether or not you wish to participate in this study. Please read this information carefully and ask as many questions as you like before deciding whether you want to take part.

- **RISKS, SIDE EFFECTS AND DISCOMFORTS:**

This study has extremely low risks, which may include; bruising or soreness at the site of the blood draw, some leg discomfort, skin irritation or fatigue from the EECP therapy.
If you experience any chest pain, shortness of breath, abnormal heart beat, lightheadedness or dizziness you should stop your activity.

- **INJURY**

Should inadvertent damage or injury result from your participation in this study, there are no designated funds provided for subsequent medical care or compensation by either the investigator, Michigan State University or William Beaumont Hospital. In the event of an injury during the course of the study, emergency medical care will be available at William Beaumont Hospital. In this event you or your insurance provider will be billed for this care. However, you do not waive any legal rights by signing this consent form.

- **COMPENSATION:**

There will be not financial compensation for this study.

- **BENEFITS:**

Participation in this study may benefit other patients with heart disease. The information gathered from this study will help doctors and therapists at EECP centers better understand the potential effects of this therapy. Furthermore, your results of all testing will be available to you.

- **ECONOMIC CONSIDERATIONS:**

There will be no payment or costs for the procedures involved in this study, which include; blood draws, Doppler studies or the use of a pedometer. EECP is considered routine care and your insurance company will be billed accordingly.

- **CONFIDENTIALITY, DISCLOSURE AND USE OF YOUR INFORMATION:**

Your privacy will be protected to the maximum extent allowable by law. Any publication or presentation of the results of this study will not identify you personally. Furthermore, your records and test results specific to this research project will only be available to the investigators of this study and will not be shared with others, except at your approval or request.

- **STOPPING STUDY PARTICIPAION:**

Your participation is voluntary and you may choose not to participate or withdraw from the study at any time without penalty or loss of benefits to which you are otherwise entitled, or without jeopardizing your medical care by your physician at William Beaumont Hospital. However, if you do not agree to sign this Consent and Authorization you will not be able to participate in this study.

- **CONTACTS:**

You may contact Liberty Van Eik, at (phone #) 248-655-5761, to answer any questions you might have about your study participation or in case you think you may have any research related injuries. If you have any questions regarding your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact – anonymously if you wish- Peter Vasilenko, Ph.D., Chair of the University Committee on Research Involving Human Subjects (UCRIHS) at Michigan State University by phone: (517) 355-2180, fax: (517) 432-4503, e-mail: ucrihs@msu.edu, or regular mail: 202 Olds Hall, East Lansing, MI 48824. You may contact the William Beaumont Hospital Institutional Review Board (Human Investigation Committee) Chairman at (248) 551-0662.

- **STATEMENT OF VOLUNTARY PARTICIPATION:**

If you agree to join this study, please sign your name below. Agreement and signature of this consent form acknowledges that you have had the procedures in this study explained to you, including the inherent risks and discomforts.

_____

Subject's signature

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Signature of investigator

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Date

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