

Effects of enhanced counterpulsation on vascular cell release of coagulation factors

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BACKGROUND: Enhanced external counterpulsation (EECP), a noninvasive treatment for patients with angina pectoris, provides long-term benefits of decreased anginal frequency and improved exercise tolerance. Previous studies have suggested that shear stress may result in angiogenesis and alter endothelial hemostatic factor release. Whether EECP therapy effects an alteration in endothelial cell proliferation and function remains unclear. The level of vascular endothelial growth factor (VEGF) and four other endothelial hemostatic factors (tissue plasminogen activator, plasminogen activator inhibitor-1, von Willebrand factor, and D-dimers) were measured in patients before and after 35 hours of EECP treatment.

METHODS: Plasma levels of endothelial growth and hemostatic factors were assessed using the standard enzyme-linked immunosorbent method.

RESULTS: No significant difference in the hemostatic factors and VEGF after EECP treatment was revealed; there was a trend toward an increase in VEGF levels posttreatment.

CONCLUSIONS: Vascular endothelial cells play a critical role in the regulation of coagulation because they control the expression of tissue plasminogen activator, plasminogen activator inhibitor-1, von Willebrand factor, and D-dimers. Our results suggest EECP may not play a role in controlling coagulation in patients with coronary artery disease through release of endothelial hemostatic factors. Although there was a tendency for increased VEGF release, larger studies are necessary to confirm these observations. (Heart Lung® 2005;34:252–6.)

Recent medical literature has determined that the vascular endothelium is a multifunctional organ essential to vascular physiology, not the simple diffusion barrier it was once thought to be. These multiple functions include serving as a selective permeability barrier, the expression of cytokines and growth regulatory agents, the modulation of vasomotor tone, and hemostatic balance.¹⁻³ The endothelium lines the vascular wall and defines the intravascular and extravascular compartments and can interact with the circulating blood and the surrounding smooth muscle cells. Sensing biochemical signals from the blood cells, the endothe-

lium reacts to these stimuli by releasing soluble products that affect the function of the cardiovascular system.¹

Hemodynamically, enhanced external counterpulsation (EECP) acts like intraaortic balloon counterpulsation by augmenting diastolic blood flow in multiple vascular beds, including the coronary arteries, and by reducing cardiac afterload.⁴ Endothelial function has been demonstrated to improve after a course of EECP therapy.⁵ In addition, EECP therapy has been associated with the release of growth factors, such as vascular endothelial growth factor (VEGF), that promote the formation of collaterals in the coronary circulation.⁶ Finally, EECP therapy may result in a “training effect” by decreasing peripheral vascular resistance in the same manner as physical exercise.⁷

Normal endothelium is nonthrombogenic. It does not permit platelet activation and adhesion, it does not activate circulating coagulation factors, and in the event of a clot it promotes the anticoagulation agents of fibrinolysis. Circulating blood is in

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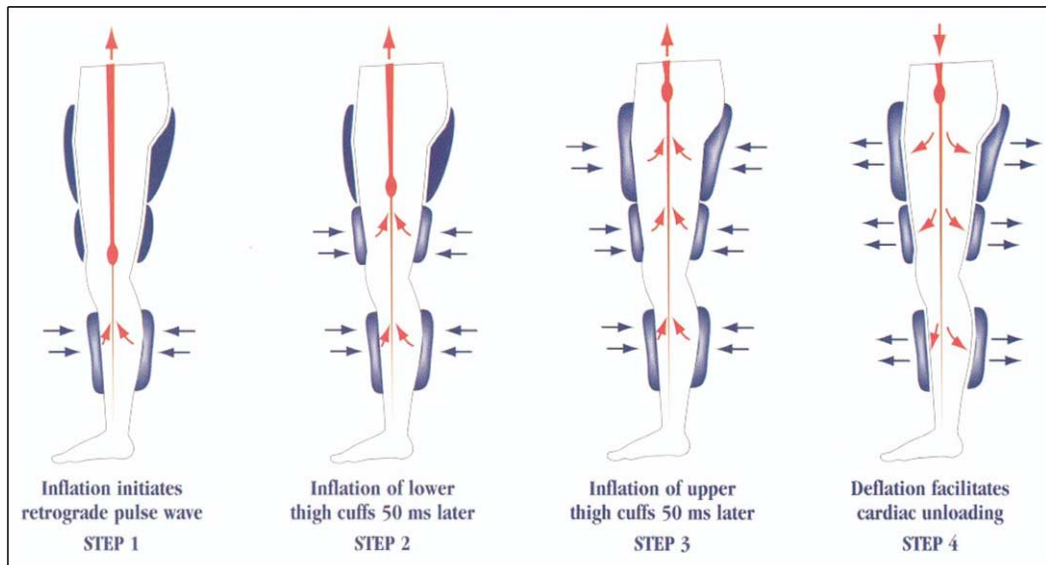


Fig 1 Principles of enhanced external counterpulsation (EECP). Series of three cuffs inflate at the calf, thigh and buttock level timed with the cardiac cycle.

dynamic equilibrium between coagulation and fibrinolysis. As with the coagulation system, fibrinolysis is the end result of an enzymatic cascade. The ultimate product of the pathway is plasmin, which degrades fibrin in thrombi. The plasmin digests the thrombi into fragments composed of D domains (D-dimers). Plasmin is an enzyme derived from an inactive precursor plasminogen that is bound to fibrin and actually forms an integral part of the thrombus. Tissue plasminogen activator (tPA), itself a protease synthesized and stored by and released endothelial cells, converts plasminogen to plasmin.

The system of fibrinolysis is regulated by several mechanisms. One significant mechanism is the control of tPA by plasminogen activator inhibitor (PAI)-1. Activated by plasmin, PAI-1 binds to plasmin and inactivates it. PAI-1, released from the endothelium and activated platelets, inactivates tPA.¹⁻³

The von Willebrand factor (vWF), another hemostatic factor synthesized by the endothelial cells, mediates platelet-to-platelet interaction and platelet vessel wall interaction. Research indicates that plasma concentration of vWF has been proposed as an indirect indicator of thrombosis and atherosclerosis.²

The endothelial cells are also responsible for the synthesis and release of growth factors such as VEGF, which stimulates angiogenesis. High levels of VEGF may be an indicator of collateral

vessel growth in the heart. Collateral growth in the heart may lead to enhanced blood flow to the cardiac muscle, which may be beneficial to patients who have angina pectoris.³

The present study examines the effects of the endothelial function in patients with coronary artery disease (CAD) who have refractory angina. EECP may affect plasma levels of tPA, PAI-1, vWF, D-dimers, and VEGF. Fig 1 shows a noninvasive treatment for patients who have angina pectoris. EECP uses an air-driven system consisting of three sets of cuffs wrapped around the calves, and lower and upper thighs, and results in enhanced oxygenated blood flow to the heart with greater efficiency during diastole. The retrograde flow provided by EECP increases both the volume of blood flow to the heart during diastole and the diastolic pressure. EECP therapy is a noninvasive option for patients with refractory angina pectoris who have failed revascularization therapy. The mechanism by which EECP works remains unclear, but it has been suggested that the shear stress provided by EECP may increase the development of collateral circulation and alter patients' hemostatic profile.

MATERIALS AND METHODS

Subjects

Thirty consecutive patients with a diagnosis of stable angina with a Canadian Cardiovascular Soci-

ety angina level of II, III, or IV were considered for enrollment. Patients with rest angina were excluded from this study. Of the 30 patients, only technically evaluable samples were studied, secondary to lack of sample pairs, refusal to give a sample, and clotting of sample. Of the cohort of 30 patients, 24 men with an average age of 66.5 ± 9.7 years were included in the study of tPA and VEGF levels. Twenty-three men were included in the study of vWF (average age 66.4 ± 9.9 years) and PAI-I (average age 66.7 ± 9.8 years) levels. Ten men with an average age of 70.9 ± 10.1 years were included in the study of D-dimer levels.

All patients went through an initial evaluation to indicate candidacy for the EECP procedure. Patients were eligible if they had evidence of CAD by one of the three criteria: (1) one or more angiographically proved stenosis greater than 70% in at least one major artery, (2) history of myocardial infarction documented by characteristic creatine kinase elevation, and/or (3) development of Q waves on the electrocardiogram or positive nuclear stress test for myocardial infarction or ischemia. The medications received by these patients were unchanged throughout the course of this study.

EECP treatment was applied to patients in 1-hour sessions for a total of 35 sessions. Patients received one treatment per day, 5 days per week.

The institutional review boards at our institution approved this study. Enrollment was conditional on subjects giving written informed consent.

Measurements of the hemostatic factors

Blood samples for plasma were collected from peripheral venous access. Blood was stored on ice in a tube containing sodium citrate until plasma was separated by spinning at $2000g$ for 15 minutes. Plasma samples were frozen at -80°C until their use for hemostatic factor level determination. Levels of tPA antigen were measured by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies. This test system measured the total amount of tPA present (free and complexed tPA, single- and double-chain tPA). PAI-I antigen concentrations in plasma were measured by an ELISA. This procedure measured the total quantity of PAI-I present (free or complexed with tPA and in active or inactive form). The vWF and fibrin D-dimer antigens were determined in duplicate by ELISAs using monoclonal antibodies from commercially available kits. The VEGF antigen concentration in the plasma was measured with an ELISA.

EECP procedure

EECP uses compressed air to inflate and deflate a series of cuffs wrapped around the patient's calves and lower and upper thighs. Pressure is applied sequentially from the patient's lower legs, lower thighs, and upper thighs and is synchronized with the cardiac cycle. Immediately after the aortic valve closes, at the onset of diastole, the cuffs sequentially inflate. This increase in blood flow is referred to as diastolic augmentation. At the onset of systole the cuffs simultaneously deflate allowing the compressed vessels to reconform, potentially reducing vascular impedance. The pressure that can be applied to the cuffs can range from 0 to 400 mm Hg. In this study the pressure ranged from 300 to 400 mm Hg. The effectiveness of EECP is measured by the ratios of peak systolic to peak diastolic pressures and the ratio of area under the systolic waveform to the area under the diastolic waveform during counterpulsation. These ratios are calculated from the signals measured by finger plethysmography.

Statistical analyses

With a paired *t* test, all hemostatic factors were measured before and after the EECP procedure with a .05 level of significance. Hemostatic factors were presented within 1 standard deviation from mean values.

RESULTS

No significant differences in hemostatic factors and VEGF after EECP treatment were revealed (Table I). There was a trend toward an increase in VEGF posttreatment (Table I). Our results suggest that EECP may not play a role in controlling coagulation in patients with CAD through release of endothelial hemostatic factors. The trend toward an increase in VEGF may be an indication of angiogenesis, but larger studies are necessary to confirm these observations.

DISCUSSION

Vascular endothelial cells play a critical role in the regulation of coagulation because they control the expression of tPA, PAI-I, vWF, and D-dimers, and the levels of such factors have been predictive in determining the severity of cardiovascular disease. Previous research has revealed that medical intervention may alter levels of endothelial hemostatic and growth factors.⁸⁻¹⁶

Yazdani et al.¹² revealed higher tPA and vWF levels in patients with unstable angina versus

Table I

Hemostatic factors and VEGF levels before and after EECP treatment

	Pre-EECP	Post-EECP	P value
VEGF (pg/mL) (n = 24)	32.13 ± 25.43	35.13 ± 28.55	NS
tPA (ng/mL) (n = 24)	12.66 ± 3.61	13.15 ± 5.34	NS
PAI-1 (ng/mL) (n = 23)	33.52 ± 18.88	29.8 ± 15.72	NS
vWF (mU/mL) (n = 23)	717.55 ± 272.47	754.23 ± 290.34	NS
D-DI (ng/mL) (n = 10)	382.98 ± 69.62	396.07 ± 80.84	NS

VEGF, Vascular endothelial growth factor; EECP, enhanced external counterpulsation; tPA, tissue plasminogen activator; PAI, plasminogen activator inhibitor; vWF, von Willebrand factor; D-DI, D-dimer; NS, not significant.

stable angina. However, after percutaneous coronary intervention, there was no significant difference in plasma levels of tPA and vWF. Therefore, medical intervention may reflect plaque reendothelialization and stabilization.

Another area of focus is the effect of exercise on endothelial function. Smith et al.¹⁰ examined the relationship between arterial compliance and endothelial-derived components of the hemostatic system, specifically vWF and tPA. Compliance of proximal and distal arteries was measured by intraarterial pulse wave analysis, left ventricular wall thickness by echocardiography, and vWF and tPA by immunoassay of plasma obtained before and immediately after maximal treadmill exercise. Compliance of distal and proximal arteries was indirectly related to levels of hemostatic factors, and this relationship was further strengthened after exercise. In addition, levels of vWF and tPA were significantly related to left ventricular wall thickness and septal thickness.¹⁰

Our results indicate that EECP treatment may not play a role in controlling coagulation in patients with CAD when a limited number of coagulation factors were measured (Table I). Plasma levels of four hemostatic factors measured before and after 35 hours of treatment remained unchanged. Unlike previous studies, all patients included in the present study had chronic stable angina, and thus no comparison of hemostatic factors associated with stable angina versus unstable angina could be made.

In addition to the four hemostatic factors measured, VEGF levels were measured before and after EECP treatment. It has been suggested that EECP may increase the development of collateral circulation with subsequent improvement in myocardial perfusion. The shear stress in coronary circulation

caused by EECP may be a potent activator of pathways involved in angiogenesis.^{17,18}

The mechanism by which EECP improves endothelial function remains largely unknown. The most prominent theory is that regulation of factors affecting endothelial functions is modulated by coronary arterial shear forces that are increased by diastolic augmentation during EECP¹⁹ and intraaortic balloon pump.²⁰ Fluid shear stresses have been shown to cause phosphorylation and thus activate endothelial nitric oxide synthetase and induce arterial vasodilatation.^{21,22} This is especially marked in patients with CAD who have low baseline nitric oxide levels. Furthermore, immediately and 1 month after a course of EECP, nitric oxide levels are significantly increased in patients with CAD.²³⁻²⁷

Vascular shear stresses also have an effect on release of endothelin-1, a potent arterial vasoconstrictor and smooth muscle mitogen. Increased coronary shear stress and mechanical pressure as provided by EECP reduced the release of endothelin-1,^{23,24} although in experimental models studies have found variable effects on the release of endothelin by mechanical forces.²⁴⁻²⁶

In addition to the effects of shear forces on vascular function and modulation of vasoactive substances, there is also evidence that it may enhance the production of angiogenic factors, which may enhance collateral vessel formation. Among patients with chronic stable angina, after a course of EECP, there was a demonstrable increase in basic fibroblast growth factor, VEGF, and hepatocyte growth factors.^{26,27} Although our results reveal a trend toward an increase in VEGF levels, no conclusion can be made about the relationship of EECP and VEGF levels.

This is one of the first studies to examine the relationship between EECP and its effect on the

vascular endothelial. Larger studies are needed to confirm these results. Limitations of the study include the following: There was a small number of patients studied (who were primarily all male), not all coagulation factors were measured, and no parallel group was used.

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