Effect of Enhanced External Counterpulsation on Inflammatory Cytokines and Adhesion Molecules in Patients With Angina Pectoris and Angiographic Coronary Artery Disease

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Cardiovascular disease is associated with chronic low-level inflammation, as evidenced by elevated circulating proinflammatory cytokines. Experimental evidence suggests that inflammation can be suppressed under conditions of high shear stress. This study was conducted to examine the effects of enhanced external counterpulsation (EECP), a noninvasive therapy that increases endothelial shear stress, on circulating levels of inflammatory biomarkers and adhesion molecules in patients with angina pectoris. Twenty-one patients were randomly assigned to either 35 1-hour treatments at cuff pressures of 300 mm Hg (EECP; n = 12) or 75 mm Hg (sham; n = 9). Plasma tumor necrosis factor–α, monocyte chemoattractant protein–1, and soluble vascular cell adhesion molecule–1 were measured before and after 35 1-hour sessions of treatment or sham. Patients in the EECP group demonstrated reductions in tumor necrosis factor-α (6.9 ± 2.7 vs 4.9 ± 2.5 pg/ml, p <0.01; −29%) and monocyte chemoattractant protein–1 (254.9 ± 55.9 vs 190.4 ± 47.6 pg/ml, p <0.01; −19%) after treatment, whereas there was no change in the sham group. Changes in soluble vascular cell adhesion molecule–1 were not observed in either group. In conclusion, 35 sessions of EECP decreased circulating levels of proinflammatory biomarkers in patients with symptomatic coronary artery disease. **(Am J Cardiol 2008;101:300–302)**

Patients with coronary artery disease demonstrate elevated levels of proinflammatory cytokines and adhesion molecules compared with levels observed in healthy controls.[1,2] Moreover, proinflammatory cytokines appear to be elevated even further in patients with angina.[3] Enhanced external counterpulsation (EECP) is a noninvasive treatment for patients with symptomatic coronary artery disease and has been shown to decrease refractory angina.[4,5] EECP significantly augments diastolic flow and increases shear stress in central and peripheral vascular beds.[6] Experimental evidence suggests that high shear stress along the blood vessel wall has a favorable effect on proinflammatory cytokine and adhesion molecule expression and signaling.[7,8] Therefore, we hypothesized that the high levels of shear stress produced during EECP would decrease circulating levels of selected proinflammatory markers and adhesion molecules in patients with angina pectoris.

Methods and results

This prospective, single-blind, sham-controlled study consisted of 21 consecutive patients with angina pectoris who were referred to EECP. All patients were recruited from the Cardiovascular Clinic at Shands Hospital at the University of Florida during a clinical screening procedure performed by a cardiologist that is mandatory for all patients referred for EECP. The study was approved by the University of Florida Health Science Center Institutional Review Board, and written informed consent was obtained from all patients. Inclusion criteria were age ≥21 years, symptoms of angina pectoris or angina equivalent present on average ≥2 times per week, and angiographic evidence of disease in ≥1 major epicardial coronary artery. Exclusion criteria included unstable angina, arrhythmia that would interfere with EECP triggering, heart failure and/or a left ventricular ejection fraction ≤30%, valvular heart disease, severe peripheral vascular disease, or uncontrolled hypertension (>180/100 mm Hg). Patients were randomly assigned to either 35 1-hour sessions of EECP at cuff pressures of 300 mm Hg (EECP; n = 12) or to a sham-EECP group (sham; n = 9) at cuff pressures of 75 mm Hg.

EECP treatment was performed at Shands Hospital at the University of Florida in Gainesville. Patients were treated for 1 hour daily on Monday through Friday for 7 consecutive weeks, resulting in a total of 35 hours of treatment. EECP involved the sequential inflation and deflation of compressible cuffs wrapped around the patients' calves, lower thighs, and upper thighs. Compressed air pressure was applied via the cuffs to the lower extremities in a sequence synchronized with the cardiac cycle via microprocessor-interpreted electrocardiographic signals. The diastolic augmentation pressure was progressively increased by increasing external compression to either 300 mm Hg (EECP) or 75 mm Hg (sham).[5] Patients were instructed to

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Table 1
Baseline patient characteristics

Variable	EECP (n = 12)	Sham (n = 9)
Age (yrs)	63 ± 11	62 ± 10
Men/women	8/4	7/2
Body mass index (kg/m^2)	29.8 ± 3.4	33.0 ± 4.2
Total cholesterol (mg/dl)	138 ± 41	142 ± 25
Low-density lipoprotein (mg/dl)	68 ± 37	72 ± 22
High-density lipoprotein (mg/dl)	45 ± 15	33 ± 7
Triglycerides (mg/dl)	123 ± 10	165 ± 76
Glucose (mg/dl)	116 ± 22	107 ± 16
Previous myocardial infarction	4	3
Multivessel coronary artery disease	10	8
Previous percutaneous coronary intervention	8	7
Previous coronary artery bypass graft	7	5
Diabetes	6	4
Hypertension	9	6
Hyperlipidemia	10	8

Data are expressed as mean ± SD.

continue their usual medications. Canadian Cardiovascular Society angina class was determined before and after the completion of the study.

Venous blood samples were collected before and after 35 sessions of EECP or sham. Plasma levels of tumor necrosis factor–α (TNF-α), monocyte chemoattractant protein–1 (MCP-1), and soluble vascular cell adhesion molecule–1 (sVCAM-1) were determined by commercially available enzyme-linked immunosorbent assay (Quantikine; R&D Systems Inc., Minneapolis, Minnesota). These specific markers were chosen on the basis of previous experimental evidence.[7,8] The intra- and inter assay coefficients of variance were 4.6% and 7.7% for TNF-α, 4.2% and 6.9% for MCP-1, and 3.4% and 6.1% for sVCAM-1, respectively. Levels of serum lipids and glucose were measured in hospital laboratories by standard and validated techniques.

Analysis of variance was used to analyze baseline group differences between the EECP and sham groups. Changes in the continuous dependent variables were analyzed by repeated-measures analysis of variance before and after 35 hours of EECP or sham. When a significant group-by-time interaction was observed, within-group comparisons between time points and between-group comparisons at each time point were performed using Tukey's post hoc analysis. All statistical analyses were performed using SPSS version 15.0 for Windows (SPSS, Inc., Chicago, Illinois). All data are reported as mean ± SD. An α level of $p < 0.05$ was required for statistical significance.

Baseline characteristics are listed in Table 1. There were no differences between the 2 groups at study entry with respect to blood pressure, drug therapy, previous cardiovascular history and/or procedures, or cardiovascular risk factors. After the intervention, patients who received EECP demonstrated improvements in Canadian Cardiovascular Society angina class (3.1 ± 0.5 vs 1.2 ± 0.4, $p < 0.01$) and reductions in anginal episodes (1.6 ± 1.4 vs 0.4 ± 0.6, $p < 0.05$) and nitroglycerin use per day (0.5 ± 0.7 vs 0.1 ± 0.2, $p < 0.05$). There were no changes in any measure of symptom reduction in the sham group.

After 35 hours of treatment, circulating levels of TNF-α (6.9 ± 2.7 vs 4.9 ± 2.5 pg/ml, $p < 0.01$) and MCP-1 (255 ± 56 vs 190 ± 48 pg/ml, $p < 0.01$) were decreased in the EECP group but had not changed in the sham group (6.4 ± 1.9 vs 6.7 ± 1.9 pg/ml, $p = 0.54$, and 270 ± 82 vs 264 ± 66 pg/ml, $p = 0.51$, respectively). There was no change for sVCAM-1 in either the EECP group (776 ± 280 vs 726 ± 278 ng/ml, $p = 0.14$) or the sham group (847 ± 177 vs 859 ± 160 ng/ml, $p = 0.81$) (Figure 1).

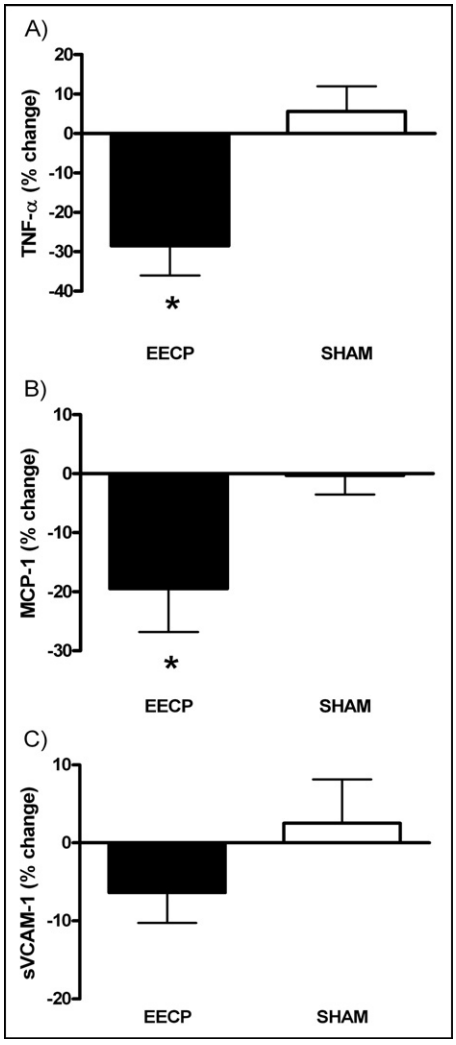

Figure 1. *(A)* Percentage change in TNF-α after 35 sessions; *(B)* percentage change in MCP-1 after 35 sessions; *(C)* percentage change in sVCAM-1 after 35 sessions. *p <0.05 versus pretreatment values. Data are expressed as mean ± SEM.

Discussion

This is the first randomized controlled study examining the effect of EECP on inflammatory and adhesion molecules in patients with coronary artery disease with refractory angina pectoris. Our results indicate that EECP has an anti-inflammatory effect in patients with angina pectoris. The percentage reduction in TNF-α (−29%) observed in the present study after EECP is similar to what has been previously reported with interventions such as exercise in patients with cardiovascular disease.[9] EECP was also effective in reduc-

ing plasma levels of MCP-1. Increased plasma levels of TNF-α and MCP-1 have been shown to predict future coronary events.[10,11] Therefore, the reductions observed in the present study may have clinical significance with regard to reducing the risk for future cardiovascular events in this patient population.

The mechanism responsible for the anti-inflammatory action of EECP is likely related to the intermittent bouts of shear stress created with each inflation-deflation cycle of the cuffs. Shear stress is a potent stimulus for the synthesis and release of endothelial-derived nitric oxide.[7] In addition to being a potent vasodilator, nitric oxide also serves an anti-inflammatory and anti-atherosclerotic role by inhibiting the expression of MCP-1 and reducing VCAM-1 expression.[12] Moreover, in arterial regions of low shear stress, there is a decrease in nitric oxide bioavailability and an upregulation of proinflammatory biomarkers.[13] Although nitric oxide production was not assessed in the present study, EECP has previously been shown to increase plasma nitrite and nitrate levels, a marker of nitric oxide production.[14,15] Decreased levels of TNF-α after EECP may have contributed to the decrease in MCP-1 levels. Chiu et al[16] showed that endothelial cells exposed to a high level of shear stress have attenuated TNF-α-induced MCP-1 expression. Although we did not observe a significant change in sVCAM-1 levels after EECP using a plasma enzyme-linked immunosorbent assay, it is possible that membrane-bound VCAM-1 levels may change in response to EECP. Unfortunately, the design of the present study did not permit the assessment of membrane-bound VCAM-1.

In conclusion, the results of the present study indicate that EECP is an effective intervention in reducing plasma levels of TNF-α and MCP-1, and these changes are paralleled by decreases in anginal symptoms. Together, these results suggest that an anti-inflammatory mechanism may help explain the symptomatic benefits of EECP. Studies involving a larger sample size and other biomarkers of inflammation are necessary to confirm our findings.

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