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Effect of Enhanced External Counterpulsation (EECP) on Inflammatory Cytokines and Adhesion Molecules in Patients with Angina Pectoris and Angiographic Coronary Artery Disease

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Abstract

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. We examined the effects of enhanced external counterpulsation (EECP), a non-invasive 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 mmHg (EECP; n= 12) or at 75 mmHg (SHAM; n=9). Plasma tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) were measured before and after 35 1-hour sessions of treatment or SHAM. Patients in the EECP group demonstrated reductions in TNF-α (6.9 ± 2.7 vs. 4.9 ± 2.5 pg/ml, $P < 0.01$; -29%) and MCP-1 (254.9 ± 55.9 vs. 190.4 ± 47.6 pg/ml, $P < 0.01$; -19%) following treatment, whereas, there was no change in the SHAM group. Changes in sVCAM-1 were not observed in either group. In conclusion, 35 sessions of EECP decreases circulating levels of proinflammatory biomarkers in patients with symptomatic CAD.

Keywords

enhanced external counterpulsation; inflammation; angina

Patients with CAD demonstrate elevated levels of proinflammatory cytokines and adhesion molecules as compared to 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 CAD 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

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circulating levels of selected proinflammatory markers and adhesion molecules in patients with angina pectoris.

METHODS

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, symptoms of angina pectoris or angina equivalent present on average of at least twice a week, and angiographic evidence of disease in at least one major epicardial coronary artery. Exclusion criteria included unstable angina, arrhythmia that would interfere with EECP triggering, heart failure and/or left ventricular ejection fraction ≤ 30%, valvular heart disease, severe peripheral vascular disease, or uncontrolled hypertension (>180/100). Patients were randomly assigned to either 35 1-hour sessions of EECP at cuff pressures of 300 mmHg (EECP; n=12) or to a sham-EECP group (SHAM; n=9) at cuff pressures of 75 mmHg.

EECP treatment was performed at Shands Hospital at the University of Florida, Gainesville, FL. Patients were treated 1 hour daily on Monday through Friday for 7 consecutive weeks, resulting in a total of 35 hours of treatment. EECP involved 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 electrocardiogram signals. The diastolic augmentation pressure was progressively increased by increasing external compression to either 300 mmHg (EECP) or 75 mmHg (SHAM).[5] Patients were instructed to continue their usual medications. Canadian Cardiovascular Society angina class was determined before and after completion of the study.

Venous blood samples were collected prior to and following 35-sessions of EECP or SHAM. Plasma levels of tumor necrosis factor alpha (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). These specific markers were chosen based on 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 measures before and after 35 hrs 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 15.0 for Windows (SPSS, Inc., Chicago, IL). All data are reported as mean ± standard deviation (SD). An alpha level of $P < 0.05$ was required for statistical significance.

RESULTS

Baseline characteristics are shown in Table 1. There were no differences between the two groups at study entry with respect to blood pressure, drug therapy, prior cardiovascular history and/or procedures, or cardiovascular risk factors. Following the intervention, patients that

received EECP demonstrated an improvement in CCS 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 nitroglycerine usage per day (0.5 ± 0.7 vs. 0.1 ± 0.2, $P < 0.05$). There were no changes in any measure of symptom improvement in the SHAM group.

Following 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 did not change 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 1A-C).

DISCUSSION

This is the first randomized, controlled study examining the effect of EECP on inflammatory and adhesion molecules in CAD patients with refractory angina pectoris. Our results indicate that EECP has an anti-inflammatory effect in patients with angina pectoris. The percent reduction in TNF-α (-29%) observed in the present study following 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 reducing 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 in regards 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 (NO).[7] In addition to being a potent vasodilator, NO also serves an anti-inflammatory and anti-atherosclerotic role by inhibiting expression of MCP-1 and reducing VCAM-1 expression.[12] Moreover, in arterial regions of low shear stress there is a decrease in NO bioavailability and an upregulation of proinflammatory biomarkers.[13] Although NO production was not assessed in the present study, EECP has previously been shown to increase plasma nitrite/nitrate levels, a marker of NO production.[14,15] Decreased levels in TNF-α following 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 following 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 from 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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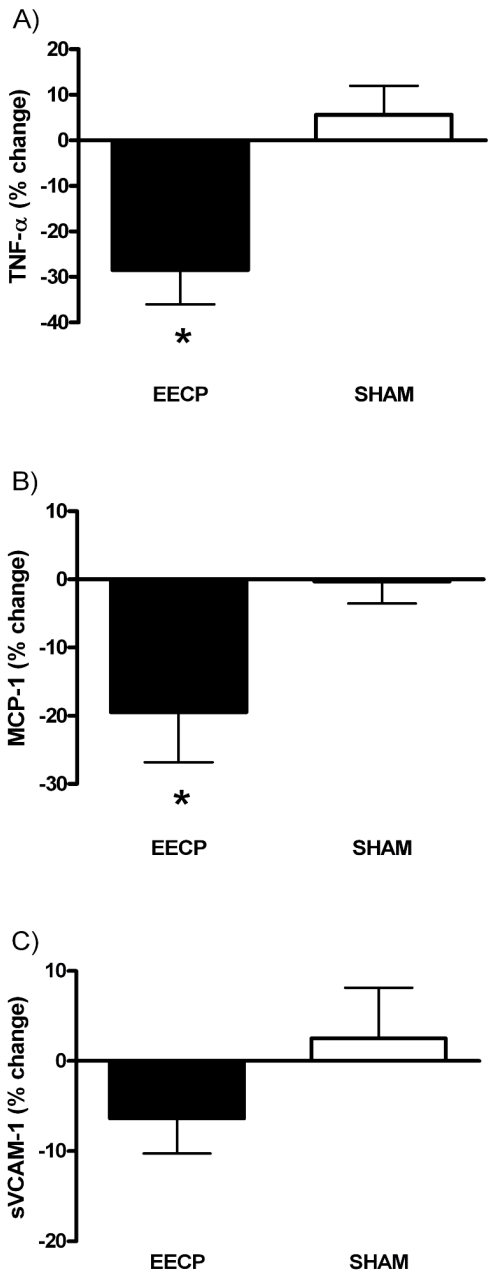

Figure 1.

A) % change in TNF-α % after 35-sessions; B) % change in MCP-1 after 35-sessions; C) % change in sVCAM-1 after 35-sessions; * $P < 0.05$ versus pre treatment values; values expressed as mean ± S.E.M.

Table 1

Baseline Patient Characteristics

Variable	EECP (n=12)	SHAM (n=9)
Age (years)	63 ± 11	62 ± 10
Male/Female	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
Prior myocardial infarction	4	3
Multivessel coronary artery disease	10	8
Prior percutaneous coronary intervention	8	7
Prior coronary artery bypass graft	7	5
Diabetes	6	4
Hypertension	9	6
Hyperlipidemia	10	8

Values are mean ± SD.