EFFECT OF ENHANCED EXTERNAL COUNTER PULSATION ON PLASMA LEVEL OF NITRIC OXIDE AND VASCULAR ENDOTHELIAL GROWTH FACTOR

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Abstract

BACKGROUND: Endothelial dysfunction contributes to the manifestation of stable and unstable coronary syndromes in patients with established coronary artery disease (CAD). Enhanced external counterpulsation (EECP) is a noninvasive therapeutic modality for patients with CAD, non responsive to medical and/or surgical treatment. The aim of this research was to determine the long-term effect of EECP on endothelial function via releasing angiogenic factors, NO (nitric oxide) and VEGF (vascular endothelial growth factor), in patients with CAD.

METHODS: The study was performed on 19 consecutive patients with ischemic coronary artery disease. All subjects were treated with EECP 1-h per day, 5 days a week, over 7 weeks (totally 35 h). Serum level of VEGF and nitrite (stable NO metabolite) concentration was measured before EECP, after 24th day, at the end of course (35th day), and at 1 and 3 months after completion of EECP treatment.

RESULTS: After 35 hours of EECP, there was a trend toward increase (31.5 ± 14.7%) in nitrite level compared with baseline (11.12 ± 3.17 vs 9.65 ± 1.36 mg/l) but it wasn't significant. Results of 1and 3 month follow-up after treatment showed that, the nitrite levels significantly increased compared with the baseline. During the course of EECP therapy, plasma VEGF levels increased progressively. Significant increase in plasma levels of VEGF were began from second sampling session of our study (24th day) and reached to maximum 3 month after EECP therapy.

CONCLUSION: In this prospective study that assessed the effects of EECP on plasma nitrite and VEGF levels, it has been demonstrated that EECP progressively increases nitrite and VEGF levels during the course of therapy. These significant changes continued 3 months after EECP therapy.

Keywords: Enhanced external counterpulsation, endothelial function, coronary artery disease

***ARYA Atherosclerosis Journal* 2009, 5(2): 61-65**

Date of submission: 27 Feb 2009, ***Date of acceptance:*** 17 May 2009

Introduction

Endothelial dysfunction contributes to the manifestation of stable and unstable coronary syndromes in patients with established coronary artery disease (CAD).[1] In addition, coronary endothelial dysfunction may lead to myocardial ischemia even in the absence of extensive obstructive CAD.[2]

Several pharmacological strategies have produced recovery of endothelial function, including ACE inhibitors, statins, and L-arginine.[3,4] However, many patients remained severely symptomatic despite extensive medical therapy.

Enhanced external counterpulsation (EECP) is a noninvasive treatment for CAD that has been approved by the Food and Drug Administration (FDA), as a therapeutic modality for patients with no response to medical and/or surgical treatment. EECP is regarded as a valuable therapeutic choice for patients with CAD and refractory angina.[5] Up to 80% of patients who go through EECP therapy have a positive clinical response.[6] EECP improves long-term prognosis in patients with coronary artery disease.[7] EECP also increases exercise capacity, improves myocardial perfusion and quality of life.[6,8,9]

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However, despite growing evidence for the clinical benefit and safety of EECP, the mechanisms contributing to its beneficial effects remain unclear.[10] It has been proposed that the favorable effects of EECP in clinical studies may be the result of new blood vessel formation (angiogenesis), and enhancement of collaterals from preexisting vessels.[12] EECP is associated with an immediate increase in blood flow in various vascular beds, including coronary arteries.[11]

This increased blood flow may lead to enhanced vascular shear stress, a key factor for endothelial production and release of nitric oxide (NO).[3,14] Furthermore, shear stress might result in the release of a variety of growth factors such as vascular endothelial growth factor (VEGF).[15]

Previous studies in animal models have been shown that EECP provided effective augmentation of angiogenesis and relevant VEGF expression.[12,16]

However, these studies have limited value in extrapolating their findings to the effects of a prolonged course of EECP because of the lack of an appropriate chronic EECP animal model and short term EECP application in them. The mechanism(s) of action, whereby EECP produces persistent long-term benefits, remains largely speculative.

The aim of this research was to determine whether treatment with EECP has any augmenting effects on two important angiogenic molecules, NO and VEGF before EECP, after 24th day, at the end of course (35th day), and at 1 and 3 months after completion of EECP treatment.

Materials and Methods

The study was approved by the ethical committee of the Isfahan University of Medical Sciences. 19 consecutive patients who were referred for EECP treatment to Chamran hospital and agreed to participate after full clarification of the risk and benefits of EECP, were prospectively enrolled in the study. Patients were required to give informed consent. Nineteen patients (13 male, 6 female) with a mean age of 61.65 ± 8.68 years, established ischemic coronary artery disease and were not amenable to standard forms of revascularization.

Exclusion criteria included unstable angina, severe valvular heart disease, uncontrolled hypertension (180/100), congestive heart failure, acute myocardial infarction < 3 months, patient with pacemaker, atrial fibrillation, left main coronary artery occlusion, and allergy to latex.

All patients underwent a standard 35-h course of EECP (1 h daily, 5 days a week, over 7 weeks).The EECP device (TS2 Vasomedical, Westbury, New York) is composed of an air compressor, a computer module, a set of cuffs, and a treatment table. Cuffs were wrapped around patient's calves, thighs, and lower buttocks.

EECP involved the sequential inflation and deflation of cuffs with air in a sequence synchronized with the patient's cardiac cycle via microprocessor interpreted electrocardiographic signals. EECP was performed at external cuff pressures of 0.35–0.40 kg/cm^2.

Venous blood samples were drawn through a 25 gauge needle immediately before EECP, after 24th day, at the end of course (35th day), and at 1 and 3 months after completion of EECP treatment. All blood samples were obtained at the same time of the day. After an immediate centrifugation at 5,000 rpm for 10 minutes at 4°C, serum was collected and stored at - 70°C until use.

Serum VEGF concentration was measured using enzyme-linked immunosorbent assay using available reagents and recombinant standards (R&D Systems, USA) according to manufacturers instruction. The VEGF assay has a minimum sensitivity of 3.0 pg/ml.

The serum levels of nitrite (stable NO metabolite) was measured using a colorimetric assay kit (Promega, USA) that involves the Griess reaction as previously described.[22] The detection limit was 0.25 μM nitrite.

Data are expressed as means ± standard deviation. Repeated-measure analysis of variance followed by paired Student's t-test, was used to detect differences in plasma nitrite or VEGF levels between baseline (before EECP) and sequential time points. SPSS v13/Win was used for data analysis.

Results

The baseline characteristics of patients are shown in Table 1. No major adverse cardiovascular events were noted during the study period.

Figure1 illustrates plasma levels of VEGF during the course of EECP therapy and 1 and 3 month following EECP termination. After 35 hours of EECP, there was a trend toward increase (31.5 ± 14.7%) in nitrite level compared with baseline (11.12 ± 3.17 vs 9.65 ± 1.36 mg/l) but it wasn't significant. Results of 1and 3 month follow-up after treatment showed that the nitrite levels significantly increased compared with the baseline 83.27 ± 29.03% and 280.80 ± 48.32%, respectively (16.47 ± 3.47 and 28.43 ± 6.04, $P < 0.05$). During the course of EECP therapy, plasma VEGF levels increased progressively (Figure 2). Significant increase of plasma levels of VEGF were began from second sampling session of our study (24th day) and reached to maximum 3 month after EECP therapy.

Table 2. Baseline Characteristics of Patients Undergoing EECP Treatment

Age (year)	61.65 ± 8.68
Female ,*n* (%)	6 (30.0)
male,*n* (%)	14 (70.0)
Cardiovascular risk factors	
Family history of premature CAD, *n* (%)	70%
Hypertension, *n* (%)	12 (60.0)
Hyperlipidemia, *n* (%)	6 (30.0)
Smoking history, *n* (%)	9 (45.0)
Diabetes, *n* (%)	5 (25.0)
Cardiac history and status	
Prior MI, *n* (%)	5 (25.0)
Prior CABG, *n* (%)	2 (10.0)
Beta-receptor antagonist	6 (30.0)
Calcium antagonists	8 (40.0)
Aspirin	15 (75.0)
Long-acting nitrates	9 (45.0)
ACE inhibitors	14 (70.0)
Lipid-lowering agents (statins)	7 (35.0)

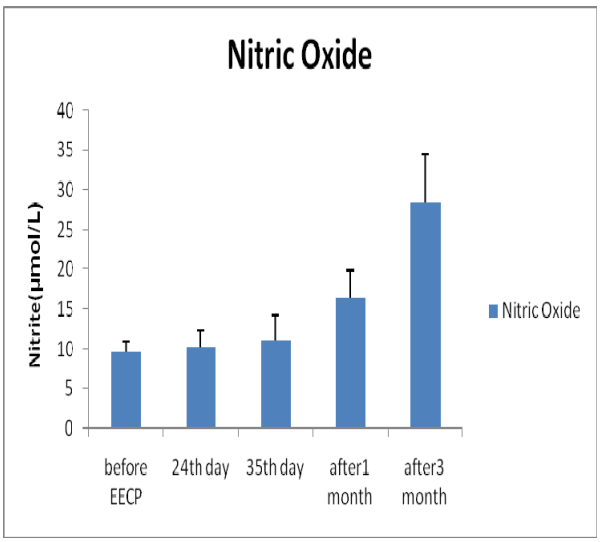

Fig 1. Plasma nitrite (stable NO metabolite) levels before, during, and at 1 and 3 months after EECP treatment ($P < 0.05$).

Discussion

In this prospective study that assessed the effects of EECP on plasma nitrite and VEGF levels, it has been demonstrated that EECP progressively increases nitrite and VEGF levels during the course of therapy. These significant changes continued 3 months after EECP therapy.

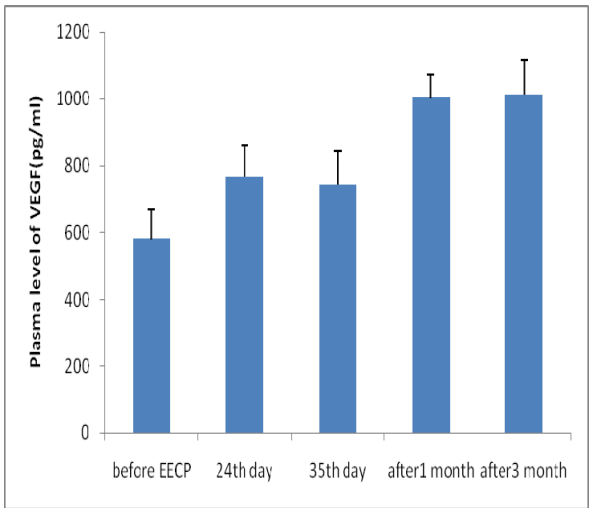

Fig 2. Plasma VEGF levels before, during, and at 1 and 3 months after EECP treatment ($P < 0.05$).

In support of our finding, EECP treatment has been shown to be associated with both an immediate and a midterm increase in plasma levels of nitric oxide in patients with CAD.[17,18] Moreover, it has been shown that EECP exerts both immediate and prolong favorable effects on peripheral endothelial function in patients with symptomatic CAD.[19]

Endothelial shear stress from increased diastolic augmentation after EECP therapy was shown to increase NO production.[20] Likewise Akhtar et al[21] showed that EECP therapy has a sustained, dose-related effect in stimulating production of NO. Endothelial NO synthase (eNOS) expression can be regulated by transcriptional activity, mRNA stability, and posttranslational modifications.[22] It has been shown that short- and long-term increases in shearstress result in enhanced NO production and eNOS bioactivity through all above mentioned mechanisms i.e. increase eNOS expression, eNOS transcript stability, and eNOS phosphorylation.[23,24] Furthermore, Levenson et al revealed that even single session of EECP increased the cGMP production in patients with coronary artery disease.[25]

VEGF and its receptors are central regulators of vasculogenesis, and angiogenesis.[26]

Sustained increase of VEGF levels in our study can also be explained by increased shear stress. Hemodynamic forces such as fluid shear stress play an active role in many physiological and pathophysiological processes of the cardiovascular system. Shear stress modifies the function of endothelial cells

throughout a complex interplay between cytoskeletal and biochemical elements and cause changes in structure, metabolism, and gene expression.[27]

It has been demonstrated that shear stress increases endothelial cell VEGF mRNA and protein expression in porcine arterial endothelial cells.[28]

In line of our results, Arora et al[23] showed a trend toward increased VEGF levels after EECP therapy in human subjects.[29] Similar finding has been reported by Chen et al[24] in the porcine model of hypercholesterolemia.[30]

By demonstrating that EECP markedly increases plasma nitrite and VEGF, our study provides evidences to support the hypothesis that EECP improves endothelial function. This hypothesis is further supported by previous studies that demonstrated by endothelium-dependent brachial artery indexes (reactive hyperemia/peripheral arterial tonometry and flow-mediated dilation) that EECP improves endothelial function in patients with symptomatic coronary artery disease, even after the first hour of therapy.[31]

Improvement in angiogenic endothelial function is considered a potential, involved in the clinical benefit associated with EECP.[32] EECP is a noninvasive treatment that is safe and effective, immediately after a course of treatment. However, the duration of benefit is less certain.[33]

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