Original Article

Long-Term Effect of Enhanced External Counterpulsation on Endothelial Function in the Patients with Intractable Angina

Mohammad Hashemi [a], Marzieh Hoseinbalam [b] and Majid Khazaei [c,*]

[a] *Department of Internal Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
[b] *Cardiac Regeneration and Neovascularization Research Group, Isfahan University of Medical Sciences, Isfahan, Iran*
[c] *Department of Physiology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

Background: **Enhanced external counterpulsation (EECP) is a noninvasive, pneumatic technique that provides favourable effects in patients with coronary artery disease. The objective of this study was to describe the long-term effect of EECP on endothelial function in patients with ischaemic cardiomyopathy.**

Method: **The study was performed in 15 patients with ischaemic cardiomyopathy. All subjects were treated with EECP 1-h per day, 5 days a week, over 7 weeks (totally 35 h). Endothelium-dependent and -independent relaxation was assessed by flow-mediated dilation (FMD) and nitroglycerine-mediated dilatation (NMD). In each patient, FMD and NMD measurements were performed before, at midcourse (day 17th) and after completion of EECP course (day 35th). In addition, FMD index was assessed 1 month after completion of EECP therapy.**

Results: **Results showed that EECP was associated with a significant improvement in FMD index after 35 hours of EECP ($10.95 \pm 4.1\%$ vs. $7.40 \pm 4.9\%$ for baseline, $p < 0.05$). NMD index didn't significantly alter during the EECP therapy. Also, 1 month after completion of EECP, FMD index returned to baseline ($7.51 \pm 4.4\%$ vs. $7.40 \pm 4.9\%$, respectively, $p < 0.05$). EECP acutely improved endothelial function in ischaemic cardiomyopathic patients. However, after 1 month completion of treatment, endothelium-dependent vasorelaxation returned to baseline.**

Conclusion: **It seems that improvement of endothelial function is not the main mechanism of long-term EECP treatment and other mechanisms should be considered.**

(Heart, Lung and Circulation 2008;xxx:1–5)

Keywords. Enhanced external counterpulsation; Endothelial function; Flow-mediated dilation

Introduction

Endothelial dysfunction has been demonstrated in asymptomatic subjects who have established cardiovascular risk factors, such as smoking, hypercholesterolaemia, diabetes, and patients with established atherosclerosis.[1] Clinical and experimental studies have shown that improvement of endothelial function by risk factor modification has led to long-term benefits in reduced cardiovascular morbidity and mortality.[2]

Enhanced external counterpulsation (EECP) was approved by the Food and Drug Administration in 1995, as a therapeutic modality for the atherosclerotic vascular disease.[3] EECP is a noninvasive, pneumatic therapy for treatment of patients with coronary artery disease. EECP provides symptom relief and improves long-term prognosis in patients with coronary artery disease. [3,4] EECP also increases exercise capacity,[3] improves myocardial perfusion[5] and quality of life.[6]

Although the exact mechanism of the beneficial effect of EECP is unclear, evidences suggest that improvement of endothelial function represents an important mechanism for favourable clinical effect of EECP.[7,8] Endothelial dysfunction can be assessed clinically by impaired vasoreactivity of the brachial artery after an ischaemic stimulus with flow-mediated dilation (FMD).[9] The purpose of this study was to investigate the long-term effect of EECP on endothelium-dependent vasorelaxation in patients with intractable angina.

Methods

Patients and Study Protocol

The study was approved by the ethical committee of the Isfahan University of Medical Sciences. All patients who were referred for EECP treatment because of refractory angina were prospectively enrolled in the study. All

Received 16 December 2007; received in revised form 4 February 2008; accepted 6 February 2008

* Corresponding author. Tel.: +98 311 7922407.
E-mail addresses: Khazaei@med.mui.ac.ir, majkhazaei@yahoo.com (M. Khazaei)

1443-9506/04/$30.00
doi:10.1016/j.hlc.2008.02.001

Please cite this article in press as: Hashemi M, et al. Long-Term Effect of Enhanced External Counterpulsation on Endothelial Function in the Patients with Intractable Angina. *Heart Lung Circulation* 2008, doi:10.1016/j.hlc.2008.02.001

patients gave written informed consent after full explanation of the purpose, nature and risk of procedure. Fifteen subjects (12 male, 3 female) with a mean age of 63.93 ± 8.68 years with an established ischaemic coronary artery disease, which were not amenable to standard forms of revascularisation, were considered for enrolment. Exclusion criteria included congestive heart failure, unstable angina, acute myocardial infarction < 3 months, systemic hypertension > 180/110 mmHg, severe LV dysfunction, significant renal impairment and allergy to latex.

All subjects were underwent EECP 1-h per day, 5 days a week, over 7 weeks (totally 35 h). Patients were encouraged to continue their diet, pharmacologic, and exercise regimens at a constant level during EECP treatment and 1 month after therapy. For each patient, all EECP sessions were scheduled at the same time of day in order to prevent possible effects of circadian variability of vascular reactivity. The brachial artery FMD index was studied before beginning of EECP, midcourse (17th day), at the end of course (35th day), and 1 month after completion of EECP treatment. Patient demographics were obtained at baseline. In addition, left ventricular ejection fraction (LVEF, biplance Simpson method) was determined by echocardiography (using HP sono 100 echocardiogram with 2.5–3 MHZ transducer) (Hewlett-Packard Inc., USA) according to the recommendations of the American Society of echocardiography before and after EECP treatment.

EECP Procedure

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. For each treatment, cuffs were wrapped around the calves and lower and upper thighs (including the buttocks) of the patient. Cuffs were connected to the air-compressor unit by air hoses. The EECP device inflates the cuffs with air and then deflates them in a sequence that is synchronised to the patient's cardiac cycle.

Pressure is applied sequentially from the calves to the buttocks, starting in early diastole. At the end of diastole, the compressed air is released rapidly from the cuffs to remove the externally applied pressure. EECP was performed at external cuff pressures of 0.35–0.40 kg/cm^2.

Assessment of Endothelium-dependent and -independent Vasorelaxation

Vascular responses in the brachial artery were studied by noninvasive, high-resolution ultrasound scans, using a modification of the technique described by Celermajer et al.[10] Endothelium-dependent and -independent relaxation was assessed by examining brachial artery response to shear stress-induced (endothelium-dependent) and nitroglycerine-mediated (endothelium-independent) stimuli. The subjects rested quietly for 15 min before the first scan and remained in the supine position throughout the study.

For this purpose, the brachial artery was scanned longitudinally 5–10 cm above the elbow, and the centre of the artery was identified when the clearest picture of the anterior and posterior arterial walls was obtained. In a satisfactory transducer position, a special probe holder designed specifically for the study was fixed around the arm to secure the ultrasound transducer, and it was held in the same position throughout the study. Depth and gain settings were set to optimise the images of the lumen/arterial wall interface that were magnified using a resolution box function. Arterial diameter measurements were made at the end-diastole (R-wave peak of the ECG) using electronic callipers. Four cardiac cycles were analysed, and an average of the measurements was taken.

For evaluation of shear stress-induced vasodilatation (endothelium-dependent relaxation), a pneumatic tourniquet was inflated around the arm to a pressure of 250 mmHg for 4–5 min and then rapidly deflated. The resulting shear stress-induced dilation (reactive hyperaemia) in the arm and the increased brachial artery diameter was recorded from 30 to 90 s after cuff deflation. Changes in the brachial artery diameter in response to endothelium-dependent NO-mediated vasodilatation induced by shear stress were expressed as a percentage change relative to the vessel diameter immediately before cuff inflation. After allowing 10–15 min for brachial artery recovery, another baseline scan was taken. For evaluation of endothelium-independent vasorelaxation, 0.4 mg of nitroglycerine spray (EGIS Pharmaceutical, Budapest) was administered sublingually; and 4 min later, the brachial artery was imaged. The response of the brachial artery diameter to nitroglycerine was expressed as a percentage change relative to the vessel diameter immediately before drug administration.

Statistical Analysis

SPSS 13 program (Chicago, IL, USA) was used for data analysis. Data are expressed as means ± standard deviation. Repeated-measure analysis of variance followed by the Bonferroni *t*-procedure was used to compare continuous data at different points in time. For comparison of continues data between groups, Student *t*-test was used. $p < 0.05$ was considered as significant.

Results

Patients

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

Effect of EECP on Endothelial Function

The FMD and Nitroglycerin-Mediated Dilation (NMD) measurements were done before and on the 17th and 35th days of treatment and one month after completion of procedure (Table 2). Fig.1B illustrates that during the course of EECP therapy, average FMD index was progressively increased. FMD index increased significantly in response to completion of the course of EECP, (baseline: 7.40 ± 4.9% vs. 35th day: 10.95 ± 4.1%; $p < 0.05$). However, on midcourse of EECP session (17th day), there was no significant change compare to the

Please cite this article in press as: Hashemi M, et al. Long-Term Effect of Enhanced External Counterpulsation on Endothelial Function in the Patients with Intractable Angina. *Heart Lung Circulation* 2008, doi:10.1016/j.hlc.2008.02.001

Table 1. *Baseline Characteristics of Patients Undergoing EECP Treatment and FMD Testing*

Age (year)	63.93 ± 8.6
Cardiovascular risk factors	
Family history of premature CAD, *n* (%)	80%
Hypertension, *n* (%)	2 (13.3)
Hyperlipidaemia, *n* (%)	10 (66.75)
Smoking history, *n* (%)	8 (53.3)
Diabetes, *n* (%)	5 (33.3)
Cardiac history and status	
Prior MI, *n* (%)	5 (33.3)
Prior CABG, *n* (%)	9 (60.0)
Prior PTCA, *n* (%)	6 (40.0)
LVEF, %	3 (20.0)
Beta-receptor antagonist	47.08% ± 11.6
Calcium antagonists	8 (53.3)
Diuretics (lasix)	4 (26.6)
Aspirin	11 (73.3)
Long-acting nitrates	9 (60.0)
ACE inhibitors	9 (60.0)
Lipid-lowering agents (statins)	11 (73.3)

baseline value (8.67 ± 5.5% vs. 7.40 ± 4.9%, respectively; $p > 0.05$) (Fig. 1B). Results of 1 month follow-up after treatment showed that the FMD index significantly decreased compared to the end of treatment (7.51 ± 4.4% vs. 10.95 ± 4.1%, respectively, $p < 0.05$) and there was no significant difference between follow-up time and baseline (7.51 ± 4.4% vs. 7.40 ± 4.9%; respectively, $p > 0.05$). Our results also showed that nitroglycerine-mediated dilatation (endothelium-independent relaxation) didn't change during the course of EECP therapy and follow-up assessment (Fig. 1C). Indeed, at the beginning of study, the LVEF was 47.08 ± 11.76% and showed no significant change after completion of treatment (data not shown).

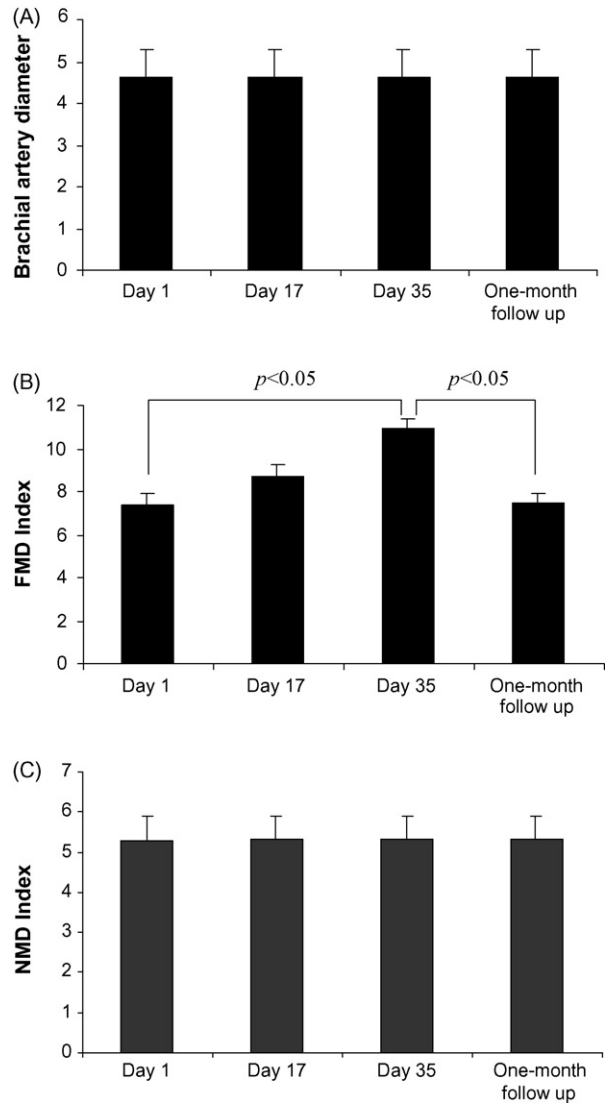

Figure 1. *Average baseline brachial artery diameter (A), peripheral flow-mediated dilatation index (FMD %) (B) and nitroglycerine-mediated dilatation (NMD %) (C) on the four study days.*

Discussion

The aim of this study was to assess the effect of long-term EECP on endothelial function in patients with intractable angina. We demonstrated that EECP didn't change endothelium-independent vasodilatation, however, it improved endothelium-dependent relaxation as measured by FMD index. We also found that 1 month after EECP, the improved endothelium-dependent relaxation returned to baseline level.

Previous studies reported that EECP treatment increased cardiac output, and sustained clinical improvement.[4,7,11] One of the mechanisms by which EECP exerts clinical benefits is improvement in endothelial function.[7,8] In this study, we used FMD index for evaluation of endothelium-dependent relaxation. We found that EECP treatment improved FMD index after 35 days. EECP-induced improvement of endothelium-dependent vasorelaxation is most probably related

Table 2. *Average Baseline Brachial Artery Diameter, Peripheral Flow-Mediated Dilatation Index (FMD, %) and Nitroglycerine Mediated Dilatation (NMD, %) on the Four Study Days*

	Before Treatment	Midcourse (17th session)	End of Treatment (35th session)	One Month Follow-up
Baseline brachial artery diameter (mm)	4.63 ± 0.66	4.61 ± 0.68	4.61 ± 0.68	4.61 ± 0.68
FMD (%)	7.40 ± 4.9	8.67 ± 5.50	10.95 ± 4.1	7.51 ± 4.4
NMD (%)	5.30 ± 0.59	5.31 ± 0.59	5.31 ± 0.59	5.31 ± 0.59

Please cite this article in press as: Hashemi M, et al. Long-Term Effect of Enhanced External Counterpulsation on Endothelial Function in the Patients with Intractable Angina. *Heart Lung Circulation* 2008, doi:10.1016/j.hlc.2008.02.001

to an increase NO production. EECP is associated with an immediate increase in blood flow in various vascular beds, including the coronary arteries.[12] The increased blood flow and shear stress have been shown to cause phosphorylation and thus activation of endothelial nitric oxide synthase (eNOS) and induce arterial vasodilatation.[13] Akhtar et al. [14] reported that EECP treatment is associated with progressive higher nitric oxide and lower endothelin-1 levels over the course of therapy. Previous studies also reported that acutely EECP treatment improved endothelial function.[8] In an experimental study in hypercholesterolaemic pigs, EECP reduced hypercholesterolaemia-induced endothelial damage, smooth muscle cell proliferation and inhibited intimal hyperplasia.[15] Levenson et al.[16] hypothesised that single session of EECP can affect plasma and platelet cGMP in patients with coronary artery disease and they showed that acute external counterpulsation increased the cGMP production. In our study, it is unlikely that improvement of flow-mediated dilation is due to smooth muscle sensitivity to NO or altering cyclic guanosine monophosphate during EECP treatment, because responses to nitroglycerine, an NO donor acting directly on smooth muscle cells, was the same as baseline.

We also demonstrated that 1 month after completion of EECP treatment, endothelium-dependent vasodilatation returned to baseline level. It is possible that it may related to decreased NO bioavailability, an effect that could result from a combination of lower NO production and/or higher NO degradation. It is indicated that cardiovascular risk factors such as ischaemic cardiomyopathy are associated with overproduction of reactive oxygen species or increase oxidative stress.[17] Thus, it suggests that control and modulation of oxidative imbalance in combination with long-term EECP program would be necessary to maintain clinical benefits. Moreover, recent data demonstrated that other mechanisms such as increase in coronary collateral perfusion and induce arteriogenesis and angiogenesis [5,18,19] may explain long-term clinical benefits of EECP.

In conclusion, we demonstrated that EECP treatment acutely improved endothelial function, as measured by FMD index. These data support a role for improvement in endothelial function as a mechanism underlying the clinical benefit associated with acutely EECP. However, after 1 month, endothelial function returned to baseline. Although in this study we didn't measure clinical or symptomatic response to EECP, however, it seems that improvement in endothelial function cannot explain long-term clinical benefits of EECP.

References

1. Clarkson P, Celermajer DS, Donald AE, Sampson M, Sorensen KE, Adams M, Yue DK, Betteridge DJ, Deanfield JE. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. *J Am Coll Cardiol* 1996;**28**:573–9.
2. Raitakari OT, Celermajer DS. Flow-mediated dilatation. *Br J Clin Pharmacol* 2000;**50**:397–404.
3. Arora RR, Chou TM, Jain D, Fleishman B, Crawford L, McKiernan T, Nesto RW. The multicenter study of enhanced external counterpulsation (MUST-EECP): effect of EECP on exercise-induced myocardial ischemia and anginal episodes. *J Am Coll Cardiol* 1999;**33**:1833–40.
4. Lawson WE, Hui JC, Cohn PF. Long-term prognosis of patients with angina treated with enhanced external counterpulsation: five-year follow-up study. *Clin Cardiol* 2000;**23**:254–8.
5. Masuda D, Nohara R, Hirai T, Kataoka K, Chen LG, Hosokawa R, Inubushi M, Tadamura E, Fujita M, Sasayama S. Enhanced external counterpulsation improved myocardial perfusion and coronary flow reserve in patients with chronic stable angina; evaluation by(13)N-ammonia positron emission tomography. *Eur Heart J* 2001;**22**:1451–8.
6. Arora RR, Chou TM, Jain D, Fleishman B, Crawford L, McKiernan T, Nesto R, Ferrans CE, Keller S. Effects of enhanced external counterpulsation on Health-Related Quality of Life continue 12 months after treatment: a substudy of the Multicenter Study of Enhanced External Counterpulsation. *J Investig Med* 2002;**50**:25–32.
7. Barsness GW. Enhanced external counterpulsation in unrevascularizable patients. *Curr Interv Cardiol Rep* 2001;**3**:37–43.
8. Shechter M, Matetzky S, Feinberg MS, Chouraqui P, Rotstein Z, Hod H. External counterpulsation therapy improves endothelial function in patients with refractory angina pectoris. *J Am Coll Cardiol* 2003;**42**:2090–5.
9. Stroes ES, Koomans HA, de Bruin TW, Rabelink TJ. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. *Lancet* 1995;**346**:467–71.
10. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;**340**:1111–5.
11. Bonetti PO, Barsness GW, Keelan PC, Schnell TI, Pumper GM, Kuvin JT, Schnall RP, Holmes DR, Higano ST, Lerman A. Enhanced external counterpulsation improves endothelial function in patients with symptomatic coronary artery disease. *J Am Coll Cardiol* 2003;**41**:1761–8.
12. Werner D, Schneider M, Weise M, Nonnast-Daniel B, Daniel WG. Pneumatic external counterpulsation: a new noninvasive method to improve organ perfusion. *Am J Cardiol* 1999;**84**:950–8.
13. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 1999;**399**:601–5.
14. Akhtar M, Wu GF, Du ZM, Zheng ZS, Michaels AD. Effect of external counterpulsation on plasma nitric oxide and endothelin-1 levels. *Am J Cardiol* 2006;**98**:28–30.
15. Zhang Y, He X, Chen X, Ma H, Liu D, Luo J, Du Z, Jin Y, Xiong Y, He J, Fang D, Wang K, Lawson WE, Hui JC, Zheng Z, Wu G. Enhanced external counterpulsation inhibits intimal hyperplasia by modifying shear stress responsive gene expression in hypercholesterolemic pigs. *Circulation* 2007;**116**:526–34.
16. Levenson J, Pernollet MG, Iliou MC, Devynck MA, Simon A. Cyclic GMP release by acute enhanced external counterpulsation. *Am J Hypertens* 2006;**19**:867–72.
17. Valgimigli M, Merli E, Malagutti P, Soukhomovskaia O, Cicchitelli G, Macrì G, Ferrari R. Endothelial dysfunction in acute and chronic coronary syndromes: evidence for a pathogenetic role of oxidative stress. *Arch Biochem Biophys* 2003;**420**:255–61.

Please cite this article in press as: Hashemi M, et al. Long-Term Effect of Enhanced External Counterpulsation on Endothelial Function in the Patients with Intractable Angina. *Heart Lung Circulation* 2008, doi:10.1016/j.hlc.2008.02.001

18. Masuda D, Nohara R, Inada H, Hirai T, Li-Guang C, Kanda H, Inubushi M, Tadamura E, Fujita M, Sasayama S. Improvement of regional myocardial and coronary blood flow reserve in a patient treated with enhanced external counterpulsation: evaluation by nitrogen-13 ammonia PET. *Jpn Circ J* 1999;**63**:407–11.

19. Wu G, Du Z, Hu C, Zheng Z, Zhan C, Ma H, Fang D, Ahmed KT, Laham RJ, Hui JC, Lawson WE. Angiogenic effects of long-term enhanced external counterpulsation in a dog model of myocardial infarction. *Am J Physiol Heart Circ Physiol* 2006;**290**:H248–54.

Please cite this article in press as: Hashemi M, et al. Long-Term Effect of Enhanced External Counterpulsation on Endothelial Function in the Patients with Intractable Angina. *Heart Lung Circulation* 2008, doi:10.1016/j.hlc.2008.02.001