



Reduced peripheral vascular reactivity in refractory angina pectoris: Effect of enhanced external counterpulsation

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Abstract

Aims To examine if the skin microvascular bed is altered and can be modified by enhanced external counterpulsation (EECP) in patients with chronic refractory angina. **Methods** Twenty patients diagnosed with refractory angina were divided into EECP ($n = 10$) or no EECP ($n = 10$) groups. The data were compared to matched healthy subjects ($n = 20$). The cutaneous forearm microvascular blood flow was measured by Laser-Doppler flowmetry. The vascular responsiveness to iontophoretic administration of acetylcholine (ACh), sodium nitroprusside (SNP) and local skin warming were studied. Measurements of Canadian Cardiovascular Society (CCS)-class, blood pressure and plasma samples were registered. **Results** EECP patients showed reduced CCS-class compared to no EECP ($P < 0.05$). Both EECP and no EECP ($P < 0.05$) groups had decreased systolic blood pressure (SBP) as compared to SBP at baseline ($P < 0.05$). There was no difference in resting blood flow between the two refractory groups at baseline as well as after EECP and seven weeks of follow-up. Responses to heating, the responses to ACh and SNP in the cutaneous microcirculation were lower in both groups of refractory angina patients as compared to healthy subjects ($P < 0.05$). EECP patients corresponded positively to the treatment shown by reduced plasma level of soluble interleukin-2 receptor and CCS-class. **Conclusions** Refractory angina patients have reduced responsiveness in their cutaneous microcirculation to ACh, SNP and heat compared to healthy subjects. Although EECP reduced the CCS-class, this effect was not associated with improvements in responsiveness of the cutaneous microcirculation.

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1 Introduction

In health or disease, it is difficult to evaluate the state and changes in vascular function in a non-invasive manner. One such way is to study the post-occlusive response to a brief brachial artery occlusion. The method may at best provide an appreciation of the endothelial function. Another way is to study superficial nutritive capillaries in the dorsal skin of the arm by laser Doppler methodology in combination with iontophoresis of acetylcholine (ACh) or sodium nitroprusside (SNP).^[1] When ACh is administered it stimulates via muscarinic receptors the formation of nitric oxide (via nitric oxide synthase) which in turn activates guanylate cyclase in the smooth muscle cells causing

relaxation.^[2] SNP is a nitric oxide donor that can bind directly with guanylate cyclase to cause relaxation. Thus, the iontophoresis of these agents may provide information on the function of the endothelin and the vascular smooth muscle cells, respectively. Here we use this methodology in order to obtain information on the function of the vascular system in refractory angina.

Enhanced external counterpulsation (EECP) is a non-invasive counter pulsation technique used for treatment of patients with chronic stable angina pectoris^[3] refractory as defined by the European Society of Cardiology Joint Study Group.^[4] The patients do not respond to revascularization procedures such as percutaneous interventions (PCI) and coronary artery bypass surgery (CABG) and have persistence of anginal symptoms despite aggressive medical treatment. The basic principle of EECP is the diastolic augmentation of arterial pressure and lowering of systolic arterial pressure along with increasing venous return. The hemodynamic effects of EECP are similar to intra-aortic balloon pumping but the treatment yields long-lasting increases in coronary blood flow.^[5,6] EECP is used to treat cardiovascular disease

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in assisting cardiac pumping effort by augmentation of the blood pressure and blood flow during diastole, decreasing resistance to pulse pressure upon systole. This form of counterpulsation increases coronary perfusion after a series of EECp sessions. Besides the acute hemodynamic effects, the long-term improvement in coronary artery disease (CAD) symptoms have been characterised by reduced angina pectoris episodes and glyceryl trinitrate use, increased exercise tolerance, positive psychosocial effects and increased quality of life.^[7–10]

However, the mechanisms responsible for the beneficial effects of EECp remain unknown. Increased coronary blood flow with diastolic augmentation has been demonstrated with transesophageal echocardiography.^[11] Further results suggest that the EECp treated patients develop and recruit collateral coronary vessels.^[12] One study in “closed-chest infarction model” in dogs had revealed increased distribution of microvessels in the region of the heart by long-term EECp, thus supporting the hypothesis that EECp promotes collateral microvascular development.^[13] In addition, shear stress and neurohormonal changes may also play a role in the clinical improvements experienced by patients treated with EECp.^[10,14] Shear stress is the product of flow and wall pressure in any vessel. Enhanced blood flow across the endothelial cell lining of blood vessels increases endothelial shear stress,^[15] which enhances endothelial function by stimulating the release of the vasodilatory mediator nitric oxide and reducing the release of the vasoconstrictive peptide endothelin-1.^[10,12,16] The improved perfusion by EECp might, in addition, be related to release of angiogenesis factors, such as vascular endothelial growth factors, basic fibroblast growth factor and hepatocyte growth factor.^[17] Recently, it was shown that EECp improved brachial artery flow-mediated dilatation and increased nitric oxide turnover/production and 6-keto-prostaglandin F_{2α} while decreasing endothelin-1 levels.^[18]

The primary hypothesis of our study was to investigate first if the skin microvascular circulation is reduced in refractory angina pectoris patients, and secondly if the microvascular responses are modified by EECp treatment. A recent study on patients with Canadian Cardiovascular Society (CCS)-class III showed improved responses of the brachial artery flow-mediated dilatation.^[18] In the current study, we aim to test effects in the peripheral microcirculation in patients exhibiting somewhat worse symptoms (CCS-class III/IV).

2 Methods

The study was conducted in accordance with the Declaration of Helsinki. The study was performed at two

Departments: Enrollment and implementation of EECp therapy was done at the Department of Medicine at the Central Hospital in Kristianstad, Kristianstad, Sweden; and the blood flow measurements were made at the Clinical Research Unit, EB block at Lund University Hospital, Lund, Sweden. The study was approved by the Regional Ethical Review Board (LU 410/2007). All patients suitable for this study approved their inclusion and signed an informed consent.

2.1 Subjects

The study involved 20 patients (17 male, 3 female, 48–83 years of age) diagnosed with stable refractory angina pectoris and CCS-class III despite optimized medical (pharmacological) and invasive therapy. Medical therapies include the maximal tolerated use of anti-angina medications (long and short-acting nitrates, β -adrenoceptor antagonists or calcium channel blockers). The patients were referred for EECp therapy after a specialist cardiologist round, due to presence of angina pectoris refractory to a combination of medical treatment and further revascularization procedures such as CABG or PCI. All patients had angiographically stated coronary stenosis (> 50%) involving at least one major coronary artery. Subjects with unstable angina, acute myocardial infarction less than three months, systemic hypertension more than 180/100 mmHg, permanent pacemaker that would interfere with EECp triggering, clinically evident peripheral vascular disease, deep vein thrombosis, phlebitis or abdominal aortic aneurysm or skin ulcers were excluded from the study.^[19]

The study was non-blinded on two arms: All patients with refractory angina pectoris waiting for EECp treatment were assigned to enter the EECp group or a second group which served as controls and then given EECp at the conclusion of the study. These two groups were perfectly matched as seen below. Assignments were randomly made by a coordinator at the Department of Medicine at the Central Hospital in Kristianstad. The coordinator was not involved in any further contact with the included patients. Data on demographics and blood samples were taken prior to intervention. The investigators of the study obtained medical history, CCS angina level^[20] coronary disease status and medication from the patients when entering the study. Laser Doppler iontophoresis and blood flowmetry (except blood samples and CCS classification) were performed by the same investigator in a standardized manner with the patient relaxing in supine position in an examining room after a rest period of 10 min. Data were collected and analyzed at the first visit for both groups (EECP and no EECp). Data were then collected after a full 35-hour course

of treatment in the EECP group or after a 7-week period in the no EECP group. In the EECP group, treatment was carried out within one week after inclusion in the study. In the other group, the EECP treatment was performed as soon as the second measurement, 7 weeks from baseline, was implemented. If any of the patients in the no EECP group showed symptoms of increased degree of angina pectoris during the study period, they were excluded from the study and immediately treated with EECP. The data were compared to a group of age matched healthy controls ($n = 20$): female 12, male 8 and 39–77 years of age randomly drawn from the regional population register and invited to participate in the study.

2.2 Clinical parameters

Hemodynamic measurements consisted of arterial blood pressure and heart rate. Blood pressure was measured non-invasively in supine position from the upper left arm with the cuff inflated at heart level. Blood pressure was taken after the iontophoresis investigation. The patients had then been resting for about one hour. The diastolic value was accepted as Korotoff's phase V. The blood pressure was measured by the same investigator three times during visit 1 and visit 2. Arterial blood pressure (systolic and diastolic) and heart rate were measured at baseline with follow-up after EECP treatment or after a 7 week period in the no EECP group.

2.3 Blood analysis

Blood samples were taken and analyzed at the Department of Clinical Chemistry, Central Hospital of Kristianstad, Kristianstad, Sweden or for interleukins at the Clinical Immunology laboratory at Lund University Hospital, Lund, Sweden. All blood samples were collected from peripheral venous access and measured by validated techniques.

2.4 Blood flow measurements and Iontophoresis

The PeriFlux System (Perimed, Järfälla, Sweden) was used to measure cutaneous blood flow. With this non-invasive system, laser-generated light is guided to the skin by a fibre optic probe. The light reflects moving blood cells in the superficial cutaneous microvessels and undergoes a shift in frequency that is proportional to the number and velocity of the moving blood cells. The Doppler effect is then used by the system in order to estimate blood flow. The patient rested at least 10 minutes before commencing with ambient room temperature of 22°C– 23°C (remained constant throughout the study). The laser-Doppler probe and the drug delivery systems were attached to the forearm. Protocol has been established previously.^[1] The chamber was filled with ACh (2% dissolved in MilliQ water; Sigma, St. Louise, MO,

USA). Skin temperature was noted and basal blood flow was registered for two minutes. Following this, ACh was injected by iontophoresis (0.2 mA at the anode for one minute). The stimulation was repeated five times with one minute rest. SNP (1% dissolved in MilliQ water; Sigma, St. Louise, MO) was applied to a new chamber (0.1 mA at the anode). The current was switched on for one minute to a total of four periods with one minute rest. Finally, the heat response was measured. A probe was heated to 44 °C and the increase in blood flow was registered for 10 min, in order to determine the maximum vasodilation possible under these experimental conditions.^[21,22] Patients refrained from long lasting nitrates, caffeine and smoking for six hours before the blood flow measurements.^[1]

2.5 EECP technique

EECP (Vasomedical Inc. Täby, Sweden) is based on the concept of counterpulsation and consists of three pairs of inflatable cuffs applied to the lower extremities. A computer activates cuff inflation and deflation using the electrocardiogram (ECG). The EECP therapist sets cuff inflation and deflation timing to the cardiac cycle escorted by the finger plethysmogram waveform. Cuffs are timed to inflate sequentially (applying 260 mmHg of external pressure) just after the onset of diastole, and then deflate simultaneously prior to the onset of systole. The instantaneous and simultaneous deflation of cuffs during systole enhances systolic unloading and reduces cardiac workload by reducing peripheral vascular resistance.

2.6 Statistical analysis

Group data is presented as mean \pm SE and 95 % confidence intervals (CI) were calculated. Visit 1 and visit 2 values were analyzed by the paired student *t* test. Fisher's exact test or the Chi(2) test was used when comparing baseline characteristics between EECP patients and no EECP patients. Differences between clinical characteristics, blood samples and blood flow responses were evaluated and analyzed by unpaired *t* test for two group comparisons and one-way analysis of variance (ANOVA), Dunn's *post hoc* test was used when comparing more than two groups. All calculations and statistics were performed by using the software program GraphPad Prism 4.0. Statistical significance was accepted when $P < 0.05$.

3 Results

All patients in the EECP group and in the no EECP group completed the study regime, and there were no adverse cardiovascular events. Tables 1 and 2 contain the patient

Table 1. Demographics of the subjects.

	EECP (n = 10)	No EECP (n = 10)	Healthy control	P-value
Age, range (years)	67 (57–83)	64 (48–77)	55 (38–81)	0.69
Gender (men/women)	8/2	9/1	8/12	0.012*
Co-existing disease				
Hypertension	2	3		0.61
Diabetes mellitus	3	5		0.36
Coronary artery disease factors and revascularization status				
CAD diagnosis, range (years)	16 (5–25)	13 (3–28)		0.51
Prior myocardial infarction	5	5		1.0
Prior revascularization	10	10		1.0
Pharmacological treatment				
Beta-adrenoceptor antagonists	8	9		0.53
Calcium channel blockers	4	4		1.0
Long-acting nitrates	10	7		0.060
RAAS-blockade	5	6		0.65
Diuretics	5	5		1.0
Lipid lowering agents	9	10		0.31

EECP: enhanced external counterpulsation; CAD: coronary artery disease; RAAS-blockade: renin-angiotensin-aldosterone system blockade; * $P < 0.05$.

Table 2. Blood pressure and analysis of the refractory angina pectoris subjects at the start (visit 1) and at the end of the study (visit 2).

Units	Visit 1				Visit 2			
	EECP, n = 10	95% CI	No EECP, n = 10	95% CI	EECP, n = 10	95% CI	No EECP, n = 10	95% CI
BMI, kg/m ²	28.9	23.3–34.3	30.9	28.1–33.7	28.4	23.1–33.8	30.4	28.2–32.7
SBP, mmHg	131	118–143	138	120–156	123	113–132	130	115–145
DBP, mmHg	70	64–76	76	64–87	69	65–74	73	65–80
Pulse, frequency/min	65	58–71	69	54–84	63	55–72	67	58–76
Uric acid, $\mu\text{mol/L}$	330	244–416	382	283–482	324	229–419	383	331–434
NT-proBNP, ng/L	422	59–785	330	12–649	459	75–844	229	51–406
Hemoglobin, g/L	135	127–143	139	132–145	138	128–148	141	134–148
Sodium, mmol/L	139	137–141	141	138–144	142	139–145	142	140–144
Potassium, mmol/L	4.1	4.0–4.2	4.2	4.0–4.4	4.1	3.9–4.3	4.1	3.8–4.4
Creatinine, $\mu\text{mol/L}$	85	68–102	93	83.1–103.2	85	67–103	91	80–102
CRP, mg/L	5.7	1.0–10.4	2.9	2.1–3.7	1.9	1.1–2.8	2.6	1.4–3.8
HbA1c, %	6.1	4.3–7.9	5.9	4.9–6.9	5.8	4.3–7.4	5.6	4.6–6.6
Homocysteine, $\mu\text{mol/L}$	13.7	10.5–17.0	14.4	9.3–19.6	13.1	9.6–16.7	11.4	7.5–15.2
TNT, $\mu\text{g/L}$	0.012	0.007–0.017	0.019	0.008–0.029	0.017	0.009–0.024	0.012	0.007–0.017
Cholesterol, mmol/L	4.3	3.1–5.4	3.3	1.8–4.8	4.1	3.0–5.1	3.9	3.1–4.6
Triglycerides, mmol/L	2.3	0.8–3.9	2.7	1.6–3.8	1.9	0.9–2.9	2.4	1.6–3.1
HDL, mmol/L	1.4	0.7–2.1	1.0	0.9–1.2	1.3	1.0–1.6	1.0	0.9–1.1
LDL, mmol/L	2.3	1.4–3.1	1.9	1.3–2.5	2.2	1.3–3.0	2.0	1.6–2.5
ApoA, g/L	1.3	1.1–1.5	1.3	1.1–1.4	1.3	1.1–1.5	1.2	1.0–1.3
ApoB, g/L	0.8	0.5–1.0	0.8	0.6–1.1	0.7	0.4–0.9	0.8	0.6–1.0
IL-6, ng/L	4.3	2.9–5.7	6.4*	4.7–8.2	5.3	4.2–6.4	6.4	5.2–7.7
sIL-2R, kU/L	786	322–1249	455	284–627	608 [▲]	409–808	638*	421–855

EECP: Enhanced external counterpulsation; BMI: Body mass index (weight (kg)/height²(m²)); SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BNP: Brain natriuretic peptide; CRP: Sensitive C reactive protein; HbA1c: Blood glucose levels in Glycated Hemoglobin A1c; TNT: Troponin T; HDL: High density lipoprotein; LDL: Low density lipoprotein; ApoA: apolipoprotein A1; ApoB: apolipoprotein B; IL-6: interleukin 6; sIL-2R: soluble Interleukin 2 Receptor. * $P < 0.05$, EECP vs No EECP, [▲] $P < 0.05$, before EECP vs after EECP.

descriptive characteristics and laboratory profiles. None of the patients in the group of refractory angina pectoris but no EECP were excluded due to worsened CCS-class. They underwent EECP treatment as planned at the end of the study. Cardiovascular medications remained unchanged in both groups during the duration of the study.

3.1 Basic data

Patients treated with EECP showed a significant reduction in CCS-class, compared to CCS-class at baseline ($P < 0.05$). This was not seen in the no EECP group ($P = \text{NS}$, Figure 1).

Systolic blood pressure (SBP) did change in the studied

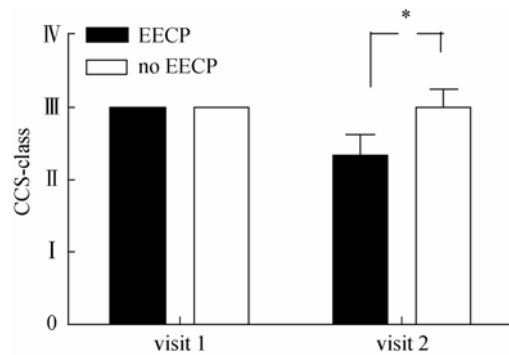


Figure 1. Comparison of the mean difference in angina measured by Canadian Cardiovascular Society (CCS) angina class after enhanced external counterpulsation (EECP) therapy and pharmacological treatment only (control) before and after EECP treatment/7 weeks. CCS-class I = Ordinary physical activity does not cause angina. CCS class-II = There is a slight limitation of ordinary activity. Angina may occur with walking more than two blocks, in the wind or under emotional stress. CCS class-III = There is a marked limitation of ordinary physical activity. Angina may occur after walking one block. CCS class-IV = There is inability to carry on any physical activity without discomfort; angina may be present at rest. All values are presented as mean \pm SE. * $P < 0.05$.

refractory groups. In the EECP group the SBP decreased from 131 ± 16 mmHg to 123 ± 13 mmHg after treatment ($P < 0.05$). There was also a decrease in SBP in the no EECP group (138 ± 17 to 130 ± 20 mmHg, $P < 0.05$).

Patients in the group not receiving EECP had a significantly higher level of blood interleukin-2 receptor (sIL-2r) concentration at the second measurement compared to the EECP treated patients ($P < 0.05$). In the no EECP group, sIL-2r was significantly increased from baseline 455 ± 185 to 638 ± 282 kU/L (kilo units/ litre) at the 7 weeks of follow-up ($P < 0.05$). This was not seen in the EECP group. In contrast to the no EECP group, sIL-2r decreased after EECP treatment from 786 ± 603 kU/L to 608 ± 261 kU/L ($P < 0.05$). Six patients treated with EECP corresponded positively in reducing the plasma level of sIL-2 receptor at the second visit. This reduction was paralleled by decreases in angina status measured by CCS-class. One patient reduced one CCS-class but increased the concentration of sIL-2r in the blood. Another patient increased both the CCS-classification and the amount of sIL-2r in the blood sample at the second measurement. Finally, two patients had no relief in the angina pectoris frequency but decreased the level of blood sIL-2r concentration at visit 2. Remaining blood samples did not differ between the two studied groups of patients (Table 2).

3.2 Laser Doppler flow and iontophoresis

The temperature of the skin was stable throughout the recording period in all groups. The laser Doppler Flow method is semi-quantitative and the data is presented as percentage change compared with the baseline perfusion value (PU). There was no difference in resting blood flow between the two groups of refractory angina patients (EECP and non EECP) at baseline during visit 1 as well as after EECP treatment, respectively at 7 weeks of follow-up (visit 2), or compared to that of no EECP.

Administration of ACh or SNP resulted in a local increase in PU that amounted to 1150% and 725% in healthy controls, respectively. These values agree well with our previous experiences.^[1] In the two groups of refractory angina pectoris, these responses were significantly lower, 694% and 486%, respectively, as compared to healthy controls ($P < 0.05$ for both). After this one group of patients got 35 treatments with EECP while the other group was only monitored. The EECP treatment did not alter the cutaneous blood flow responses to ACh or SNP in refractory angina pectoris.

At the first examination of the microvascular blood flow, responses in the two angina pectoris groups were significantly lower compared to the response seen in the healthy controls ($P < 0.05$). The response to the local heating ($+44^{\circ}\text{C}$) in the two groups of refractory angina patients was also lower compared to that of the healthy subjects ($P < 0.05$, Figure 2).

4 Discussion

The present study is the first to show that the microvascular dilator responses to cutaneous ACh, SNP and local heating are all reduced in patients with refractory angina pectoris compared to age matched healthy controls. This suggests a general reduction in the vascular reactivity of the microvasculature in refractory angina pectoris. Other studies of man have revealed reduction in cutaneous vessel responses in hypertension,^[22] in aging,^[1] and in congestive heart failure.^[23] Thus, the present method is well suited to monitor alterations in the peripheral vascular activity in one type of peripheral vascular beds. Interestingly, therapy resulting in better status of congestive heart failure patients was found to improve the vascular responses to ACh and SNP.^[23] Here we found that although EECP results in significantly reduced CCS-class, there was no improvement in microvascular responses. In support, a previous study has examined the skin microcirculation of healthy subjects compared to older patients with CAD before and after one hour of EECP. Reportedly the peripheral circulation was

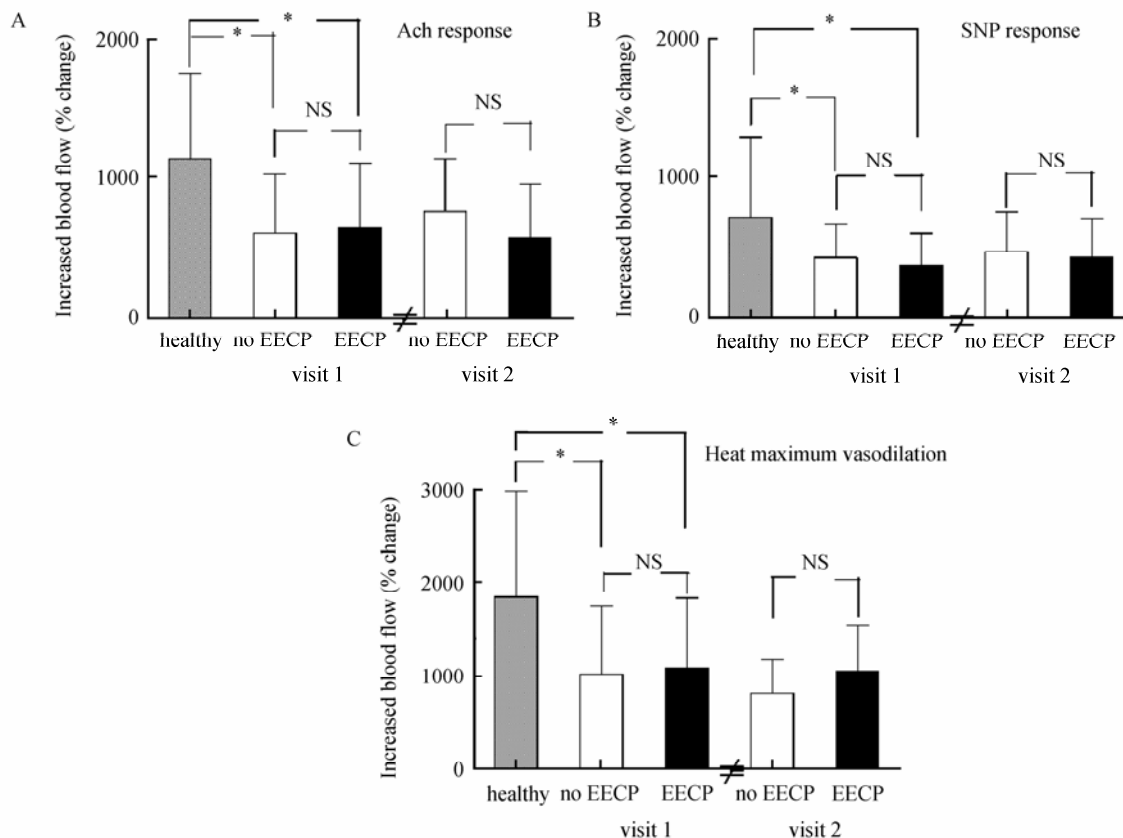


Figure 2. Microcirculatory responses to ACh (A) and SNP (B) induced increase in cutaneous blood flow in the two refractory groups (EECP and control) and in healthy control. Figure 2C indicates percent change of microcirculatory response to local heating (+44°C) in the refractory group (EECP and control) and in the healthy control. All values are presented as mean \pm SE. * $P < 0.05$, NS = not significant. ACh: acetylcholine; SNP: sodium nitroprusside; EECP: enhanced external counterpulsation.

improved with superficial skin blood flow, transdermal oxygen and carbon dioxide pressure.^[24] Our results suggest that the vasodilator capacity in patients with refractory angina pectoris is associated with reduced responsiveness in the peripheral microcirculation but the better status was not within the relatively short time period of up to three months not associated with better skin blood flow reactivity. The difference is probably due to methodological issues.

Although the CAD might be associated with a general reduction in the circulation recent data suggest that brachial artery flow-mediated dilatation is improved by EECP.^[18] This would suggest that EECP has effects primarily on the more central parts of the circulation.

Flow-mediated dilation in the brachial artery is an often used method for assessing endothelial function in humans. However, there is controversy to whether endothelial function in the brachial artery is a manifestation of the status of coronary microcirculation.^[25,26] Considering the differences in the microvascular architecture, blood flow pattern and metabolic regulation between peripheral and coronary

circulation, it could be assumed that reduced CCS-class related to EECP treatment is not associated with an improvement in responsiveness of the cutaneous microcirculation but coronary endothelial function. Further, we can not establish that the vasodilator response to ACh, SNP and heating is an index of coronary microcirculation.

The results of this study confirm that EECP treatment improves the CCS-class in patients with refractory angina pectoris. This is in accordance with previous studies.^[3,8,9,27-29] In addition, the current study compared treated patients with a parallel group of matched subjects with refractory angina pectoris waiting for EECP treatment. A significant reduction in angina pectoris was measured by CCS-class in the EECP group compared to the no EECP group. This symptom relief has earlier been demonstrated.^[30] The possible mechanisms responsible for the improvement of the angina status include enhancement of the endothelial function, promotion of collateral circulation and improved ventricular function.^[31] The consequences of these mechanisms are a reduction in ischemia, which produce pain relief. EECP causes enhanced

shear stress in the coronary circulation by diastolic augmentation. This is thought to activate factors that modify endothelial functions, which may be potent activators of angiogenesis, such as endothelial growth factors.^[32] However, we can not confirm that the endothelial function was improved, at least not in the cutaneous circulation.

The significant reduction in CCS-class could be due to possible central effects. EECP did not improve the peripheral cutaneous reactivity to the endothelial vasodilator ACh or the smooth muscle cell specific dilator, SNP. In this study, both these mechanisms were shown to be reduced in refractory angina pectoris patients as compared to age matched healthy controls and patients not modified by EECP. Hilz *et al.*^[24] reported enhanced skin oxygenation on carbon dioxide clearance during EECP treatment, as an acute effect following just one brief session of EECP for five minutes. The study did not give information if it persisted. A typical course of EECP therapy treatment (as in our case), consists of 35 hours in total.^[7,33] Hashemi *et al.*^[34] investigated the long-term effect of EECP on endothelium-dependent vasorelaxation in patients with refractory angina pectoris by noninvasive, high-resolution ultrasound scans. Their data suggest improvement in endothelial function, as a mechanism underlying the clinical benefit associated with EECP. After one month, endothelial function returned to baseline measurements. This study did not measure symptomatic response to EECP and could not explain long-term clinical benefits of EECP due to improvement in endothelial function. The medical therapy was not changed during the current study period. Since this pharmacological treatment has shown cardio protective and symptom relieving effects,^[35] it was not ethical to discontinue their medical therapy in order to exclude possible interactions with the specific therapy, EECP. The medical therapy might somehow explain the reduced cutaneous blood flow response to ACh or SNP in refractory angina pectoris patients undergoing EECP treatment. Furthermore, the presence of a matched group of patients not receiving EECP treatment supports our findings.

The study by Braith and colleagues showed that EECP improved responses to some vasodilators and reduced vasoconstrictor responses such as to that of endothelin-1.^[18] We did not study these but the parameters that were comparable are in agreement (Table 2). On the other hand, there was in the present study a reduction in sIL-2 receptor level after EECP compared to its matched no EECP group after 3 months of only symptomatic treatment where the sIL-2 receptor level was significantly increased. This result is in accordance with earlier studies investigating the effects of EECP on circulating levels of inflammatory biomarkers

in patients with angina pectoris. Cardiovascular diseases are associated with a chronic low-level inflammation.^[36] Experimental evidence suggests that shear stress in high volume along the blood vessel wall affect positively on proinflammatory cytokines.^[37] A study by Kasapis and Thompson^[38] showed that regular exercise yielded a long-term anti-inflammatory effect by improved endothelial function and reduced inflammatory markers. In a manner similar to regular physical exercise, EECP is able to increase cardiac output and blood flow. Shephard *et al.*^[39] suggested that moderate training reduce the exercise-induced suppression of IL-2 production. This study demonstrates that EECP is a treatment corresponding positively in reducing the plasma level of sIL-2 receptor. This reduction was paralleled by decreases in angina status measured by CCS-class. Thus, this may suggest a tendency toward anginal improvement via reduced inflammation. This is in agreement to a study on congestive heart failure with inflammation and B12 deficiency.^[21] Treatment with B12 supplementation normalized homocysteine, reduced inflammatory markers and improved the condition of the vascular reactivity.

4.1 Limitation to the study

The study was non-blinded on two arms. The assignments were made by a coordinator which was not involved in the further contact with the included patients. Thus, the control group was from a statistical point of view not different from the EECP group ($P > 0.05$). The healthy control on the other hand had somewhat more patients who were women. This could have skewed the data. However the results indicate a general reduction in the vascular reactivity of the microvasculature in both groups of patients with refractory angina pectoris. Thus, the lower reactivity may partly be caused by gender factors, however previous data would suggest that this is not the case. The results demonstrate that EECP is more effective in decreasing angina measured by CCS-class as compared with refractory patients only receiving pharmacological treatment. A direct comparison in a double-blinded, randomized manner is, however, required to verify this suggestion.

The study was conducted on patients with severe angina pectoris and they were all considered not suitable for any more invasive investigations or surgery. So measurements of coronary vascular function relying on invasive procedures were not possible. The laser Doppler, a non-invasive system in combination with iontophoresis of ACh or SNP was used in order to investigate the cutaneous microcirculation. The decreased angina measured by CCS-class in patients with refractory angina pectoris undergoing EECP was not

accompanied by changes in the cutaneous microvasculature. The improved response of EECP in anginal status may therefore be limited to the coronary arteries. In year 1992, Sütsch *et al.*^[40] demonstrated that the abnormal response of the microvasculature dilator stimulus in patients with microvascular angina was limited to the coronary arteries (coronary sinus blood flow was measured by the coronary sinus thermodilution technique) and did not include the skin vessels. On the other hand, Sax *et al.*^[41] used plethysmographic assessments of the forearm and showed that peripheral vascular responses correlated with those in the coronary circulation in patients with microvascular angina. Furthermore, IJzerman *et al.*^[42] reported that increased coronary heart disease score was associated with impaired skin microvascular responses, both endothelium-dependent and endothelium-independent. Which of the mechanism that is responsible for the improved anginal status measured by CCS-class in patients with refractory angina undergoing EECP can not be answered by the present study. Thus, a comparative study should be of interest.

4.2 Conclusions

Taken together, refractory angina pectoris is associated with reduced microvascular responsiveness to endothelial and smooth muscle stimulants, e.g., to ACh and SNP, as well as to local heating. However, EECP did not improve these responses compared to a control group of patients. Together with other studies it is suggested that treatment with EECP may initially mainly improve the coronary circulation.

Competing interests

The authors declare that they have no competing interests.

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