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Effect of enhanced external counterpulsation on circulating CD34+ progenitor cell subsets

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ABSTRACT

Background: Enhanced external counterpulsation (EECP) is associated with improvement in endothelial function, angina and quality of life in patients with symptomatic coronary artery disease, although the mechanisms underlying the observed clinical benefits are not completely clear. The purpose of this study was to examine the effects of EECP on circulating haematopoietic progenitor cells (HPCs) and endothelial progenitor cells (EPCs) in patients with refractory angina. We compared HPC and EPC counts between patients scheduled for EECP and patients with normal angiographic coronary arteries, with and without coronary endothelial dysfunction. We hypothesized that an increase in circulating bone marrow derived progenitor cells in response to EECP may be part of the mechanism of action of EECP.

Methods: Thirteen consecutive patients scheduled to receive EECP treatment were prospectively enrolled. Clinical characteristics were recorded and venous blood (5 ml) was drawn on day 1, day 17, day 35 (final session) and one month post completion of EECP therapy. Buffy coat was extracted and HPCs and EPCs were counted by flow cytometry.

Results: Median Canadian Cardiovascular Society (CCS) angina class decreased and Duke Activity Status Index (DASI) functional score increased significantly (both, $p<0.05$) in response to EECP, an effect that was maintained at one month after termination of treatment. Flow cytometric analysis revealed an accompanying significant increase in CD34+, CD133+ and CD34+, CD133+ CPC counts over the course of treatment ($p<0.05$). DASI scores correlated significantly with CD34+ ($R=0.38$ $p=0.02$), CD133+ ($R=0.5$, $p=0.006$) and CD34+, CD133+ ($R=0.47$, $p=0.01$) CPC counts.

Conclusion: This study shows that HPCs, but not EPCs are significantly increased in response to EECP treatment and correlate with reproducible measures of clinical improvement. These findings are the first to link the functional improvement observed with EECP treatment with increased circulating progenitor cells.

1. Background

Enhanced external counterpulsation (EECP) therapy is associated with improvements in myocardial ischaemia, angina, nitrate use [1], exercise tolerance [1–3], and quality of life [4,5] in refractory angina patients who fail to respond to conventional revascularization and aggressive anti-anginal medication. Furthermore, the report from the International EECP Patient Registry showed that EECP treatment decreased angina episodes and improved quality of life even in patients with severe left ventricular (LV) dysfunction (ejection fraction <or =35%) [6]. These beneficial effects occur early after initiation of therapy and are sustained in many patients up to 5 years later [7,8].

EECP is a non-invasive treatment for angina that uses the sequential inflation and deflation of lower extremity pneumatic cuffs to reduce left ventricular after load and augment diastolic flow and coronary perfusion pressure. While the haemodynamic effects are well-established [9], the exact mechanisms by which EECP exerts its beneficial clinical effects are unresolved. Improvement in the peripheral endothelial function of intractable angina patients as measured by reactive hyperemia-peripheral arterial tonometry after EECP has been suggested as a mechanism of action of EECP [10]. Tao and colleagues have demonstrated that EECP improves endothelium-dependent vasorelaxation in the carotid arteries of hypercholesterolemic pigs [11]. The pathways by which EECP can improve endothelial function and improve symptoms of angina are poorly understood.

We have recently demonstrated a reduction in circulating haematopoietic progenitor cells (HPCs), but not in endothelial precursor cells (EPCs) as currently defined, in the syndrome of coronary endothelial dysfunction [12]. We hypothesized that EECP, owing to its documented beneficial effects on coronary endothelial function, would have an effect on specific circulating CD34+ subsets. Therefore, circulating CD34+

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Table 1
Baseline characteristics of study cohort.

Patient cohort	Percentage (%)	Number (n = 13)
Male	100	13
Age>65 years	85	11
BMI>25	85	11
LVEF<35%	31	4
Prior CABG	85	11
Prior redo-CABG	23	3
Prior PCI	62	8
More than 1 prior PCI	46	6
Prior EECP	8	1
Prior TMR	8	1
Type 1 diabetes	15	2
Type 2 diabetes	31	4
Hypertension	92	12
Hyperlipidemia	100	13
Current smoker	0	0
Ex smoker	69	9
CRF (on dialysis)	15	2
PAD	62	8
Cerebrovascular disease	31	4

cells expressing CD45 (dim) and without evidence of CD45 expression were analyzed. As discussed previously, these subtypes are in keeping with HPCs and putative EPCs respectively [13,14].

2. Methods

2.1. Patients

Thirteen consecutive patients with chronic stable angina referred for EECP treatment were enrolled in the study. The study was approved by the Institutional Review Board of the Mayo Clinic, Rochester, Minnesota, USA, and written informed consent was obtained from all patients. All patients were symptomatic of refractory angina, had several prior cardiac events and interventions, including coronary artery bypass grafting and percutaneous coronary interventions, and were considered unsuitable for further conventional percutaneous or surgical revascularization by at least two senior interventional cardiologists (Table 1).

The patients were referred for EECP treatment because they had a chronic condition characterized by the presence of severe, Canadian Cardiovascular Society (CCS) class III or IV angina caused by myocardial ischaemia in the presence of angiographic multivessel native coronary artery disease (CAD) that could not be controlled by a combination of optimal tolerated medical therapy (Table 2), angioplasty/stent, and/or coronary artery bypass surgery. Cardiovascular medications remained unchanged during the 7-week course of EECP treatment.

Exclusion criteria included acute unstable angina, large aortic aneurysm, severe aortic valvular insufficiency, markedly irregular heart rhythm, hypertrophic cardiomyopathy, overt cardiac failure, severe uncontrolled hypertension (systolic pressure>170 mm Hg or diastolic pressure>100 mm Hg), or severe peripheral vascular disease. Patients were instructed to abstain from eating, smoking, and drinking caffeinated beverages at least 2 h before each EECP session.

Patients were interviewed before each session to obtain information on severity and number of angina episodes, amount of nitroglycerin used, and general cardiovascular function. Each patient's functional capacity was estimated at baseline, at treatment midpoint and after the full course of EECP using the Duke Activity Status Index (DASI). The DASI is a simple, easily administered, 12-item quality-of-life instrument that provides a patient's self-assessment of their functional capabilities. Original development of the DASI was correlated and validated to estimate maximal oxygen consumption measurements at peak exercise [15]. Functional capacity is assessed based on the estimated peak oxygen uptake, as determined by the following equation:

$$\text{Estimated Peak Oxygen Uptake in mL / min} = (0.43 \times \text{DASI score}) + 9.6.$$

This total score can be divided by 3.5 to estimate metabolic equivalent tasks (METs).

The control group consisted of 19 patients evaluated for chest pain by cardiac catheterization, but without findings of significant obstructive disease. These patients all underwent invasive assessment of coronary endothelial function by intracoronary acetylcholine challenge testing as described previously [16,17], and coronary endothelial function was found to be abnormal in 12 patients and normal in 7. Cell counts were analyzed from buffy coat in an identical manner to that described for the EECP patient cohort and were compared to circulating progenitor cell in this group.

Table 2
Cardiovascular pharmacotheraphy for study cohort.

Patient cohort	Percentage (%)	Number (n = 3)
Aspirin	100	13
Clopidogrel	31	4
Lipid lowering therapy	100	13
Long acting nitrate	100	13
Calcium channel antagonist	85	11
Diuretic	62	8
ACE inhibitor	46	6
ARB	46	6
L-arginine	23	3
Beta blocker	85	11

2.2. EECP therapy

All patients were treated with 35 h of EECP divided into 1-hour daily treatments over a period of 7 to 8 weeks. The EECP device (Luminair, Vasomedical Inc., NY, USA) 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 by air hoses to the air compressor unit. The EECP device inflates the cuffs with air and then deflates them in a sequence that is synchronized 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 to 0.40 kg/cm^2. Assessment of acute diastolic pressure augmentation during EECP was monitored using conventional finger plethysmography.

2.3. Flow cytometry

Mononuclear cells were extracted using density gradient centrifugation and then analyzed by flow cytometry as previously described for CD34+ $CD45_{dim}$ VEGFR2- cells (HPCs) and CD34+ CD45- VEGFR + cells (EPCs) [12].

2.4. Statistical methods

All data were stored and analyzed using JMP software. Data are presented as mean value and standard deviation for continuous variables. Comparisons between the baseline (before the first session) indices of functional capacity (DASI score, peak VO2 and total METS achieved) and those after EECP treatment (before the 35th session) were assessed using a paired 2-tailed Student t-test for paired observations. A Kruskal–Wallis test was used to analyze progenitor cell counts at four time points. Correlations between cell counts and indices of functional capacity were performed using a sum of least squares regression analysis. A p value of <0.05 was considered to be significant.

3. Results

Thirteen patients with advanced symptomatic coronary artery disease were prospectively studied (Table 1). The patient cohort was entirely of male gender with a mean age of 71 years (range 64–84 years). Patients had a long history of CAD, most had undergone multiple percutaneous coronary interventions, and the majority had undergone coronary artery bypass grafting. The patients had extensive co-morbidites including hypertension, hyperlipidaemia, chronic renal impairment (15% were dialysis dependent), diabetes mellitus and peripheral arterial disease. All patients had refractory CCS class III and IV angina and were not felt to be candidates for further percutaneous or surgical revascularisation as determined by at least two senior operators. The majority of patients were taking a combination of up to 5 anti-anginal medications (Table 2). All thirteen patients completed the entire EECP treatment, which lasted for 7 to 8 weeks (35 1-hour sessions) and experienced no serious cardiovascular events.

Thirty-five EECP sessions were associated with an improvement of one CCS class in 8 (62%) patients and by two CCS classes in 2 (15%) patients, whereas 3 (23%) patients demonstrated no change in CCS class at the end of EECP treatment. Median CCS class decreased significantly in response to EECP, an effect that was maintained at one month after termination of treatment. The mean DASI score, a measure of functional status, also increased significantly with EECP treatment (baseline 12 ± 2 vs. 19 ± 3 at 35 days), with a corresponding increase in derived maximum VO_2 (14.7 ± 0.8 vs. 18.0 ± 1.1) and metabolic equivalents or METs (3.4 ± 0.5 vs. 5.5 ± 0.7),

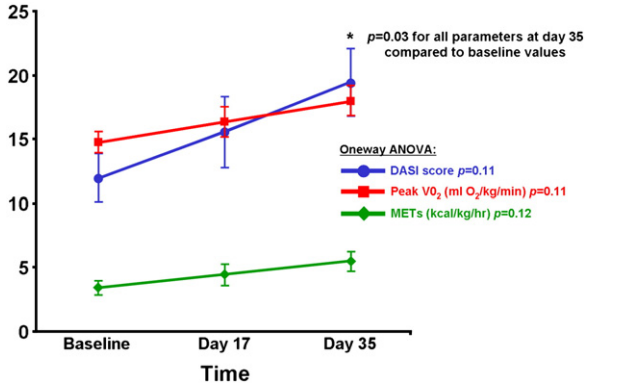

Fig. 1. Data are displayed at baseline, at 17 days and at 35 days of treatment. Statistical analysis was performed using one-way ANOVA analysis and also between baseline and 35 day values by Student t-test.

$p = 0.03$ comparing 35 day values to baseline for all parameters (Fig. 1).

One-way ANOVA analysis of circulating progenitor cell counts measured by flow cytometry revealed a significant increase in CD34 + 45_{dim} ($p = 0.03$), CD133 + 45_{dim} ($p = 0.03$) and CD34+, CD133+, $CD45_{dim}$ cells ($p = 0.02$) over the course of treatment (Fig. 2). These cells are in keeping with current definitions of circulating HPCs [13]. This finding was not observed in the case of CD34+ CD45- VEGFR2+ cells (Fig. 2) which are consistent with current definitions of EPCs [14]. Cell counts from the seven patients with normal coronary endothelial function, and twelve patients with abnormal coronary endothelial function at the time of invasive coronary hemodynamic assessment are also shown. The CD34 + 45_{dim}, CD133 + 45_{dim} and CD34+, CD133+, $CD45_{dim}$ cell counts (HPCs) of patients with both normal and abnormal coronary endothelial function, but no obstructive coronary disease differed significantly from patients recruited for EECP therapy before treatment was commenced ($p = 0.02$). However by day 17 of EECP therapy, the differences in these cell counts were no longer apparent between EECP patients and patients with coronary endothelial dysfunction but no obstructive coronary disease. These findings were also observed at day 35 of EECP therapy and one month after EECP therapy was discontinued.

CD34+, CD45-, VEGFR2+ cell counts (putative EPCs) did not change significantly over the course of EECP therapy ($p = 0.17$), and did not differ significantly from those found in patients with normal or abnormal coronary endothelial function and without significant obstructive coronary disease.

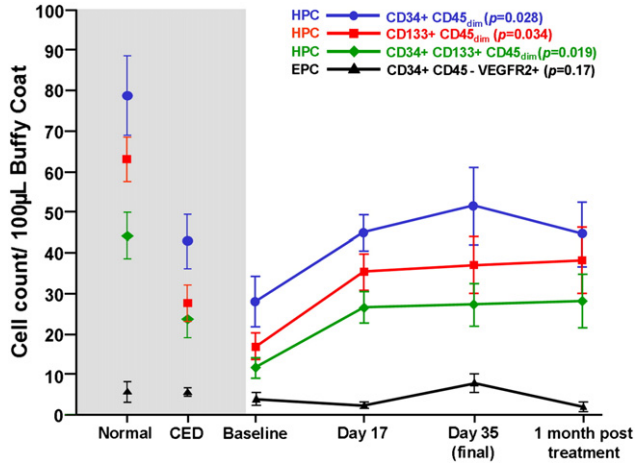

Fig. 2. Effect of EECP on CPC counts. CD34+, CD133+ and CD34, CD133 double positive cells at baseline, 17 days, 35 days and 1 month post treatment are shown (one-way ANOVA, Kruskal–Wallis test). Comparisons between baseline values and those at the other time points are also shown (Wilcoxon test). Historical control cell counts are shown to the left (shaded area) [12].

The DASI scores and the derived VO_2 and METs values also correlated significantly with the CD34 + 45_{dim} HPC cell count ($R = 0.38$ $p = 0.02$), CD133 + 45_{dim} HPC cell count ($R = 0.5$, $p = 0.006$) and CD34+, CD133+, $CD45_{dim}$ HPC cell count ($R = 0.47$, $p = 0.01$) on bivariate analysis. No significant correlation was observed between CD34+, CD45-, VEGFR2+ EPC count and DASI score ($R = 0.28$, $p = 0.14$) (Fig. 3).

4. Discussion

Since the results of the first double-blind randomized placebo-controlled multicenter trial were published (MUST-EECP [18]), EECP therapy has emerged as an effective, non-invasive, and durable therapeutic option for patients with angina and the American Heart Association recommends it as a Class IIb (Level of Evidence: B) intervention for treatment of refractory angina pectoris [19,20]. The mechanism of action and the overall effects of EECP therapy have not been fully elucidated.

Development of new functional collateral vessels by increasing plasma nitric oxide (NO) and decreasing endothelin-1 levels to the ischemic myocardium have been postulated as a mechanism of action for EECP therapy. EECP has been shown to augment plasma nitrate/nitrite production, an indirect measure of plasma NO production, to cause down-regulation of endothelin-1 levels and indeed these findings can be sustained through the course of EECP therapy [18].

EECP therapy has demonstrated improvement in endothelial release of nitric oxide resulting in improved endothelial function [18]. The mechanism of how endothelial improvement is brought about in these patients is uncertain and various theories include involvement of the NF-kappa signaling pathways, an increase in cyclic guanosine monophosphate (cGMP) [21] or an increase in vascular endothelial growth factor levels [22].

In our study we found that in patients with refractory angina undergoing EECP therapy, FACS (Flow cytometric analysis) revealed an increase in HPC counts over the course of treatment, which was statistically significant for all subtypes analyzed i.e. CD34+ $CD45_{dim}$, CD133+ $CD45_{dim}$ and CD34 CD133+ $CD45_{dim}$ HPCs by one-way ANOVA ($p < 0.05$). This increase in HPC counts remained sustained for one month after the final EECP session, and the associated clinical benefits in these patients is analogous to findings of Bonetti and colleagues, who showed that clinical and endothelial function improvements persisted at one month after EECP treatment [10].

Previous studies have shown that patients with refractory angina and multiple risk factors, as in our study cohort, have very low circulating levels of endothelial progenitor cells [23]. Patients with low circulating progenitor cell counts have a higher incidence of cardiovascular events compared to patients with higher counts. Recent studies have shown that the number of circulating progenitor cells predict severe endothelial dysfunction independent of classical cardiovascular risk factors [12,24]. Our patient cohort also demonstrated a significant and sustained improvement in DASI scores during the EECP treatment period which correlated significantly with the HPC counts. Exercise alone has been shown to increase circulating progenitor cell counts in healthy young patients but interestingly, older patients with peripheral arterial disease appear to be unable to mount a significant increase in circulating progenitors [25]. It is logical to surmise that EECP and an associated improvement in functional status leading to greater exercise capacity, together may have resulted in the significant increase in HPCs demonstrated in our patient population.

This study again demonstrates a difference in effect on circulating CD34+ subsets, in this case as a response to EECP therapy. HPC counts ($CD45_{dim}$ subset) became significantly elevated from baseline early in the course of treatment and this effect was maintained for the remainder of the treatment course and even one month afterwards.

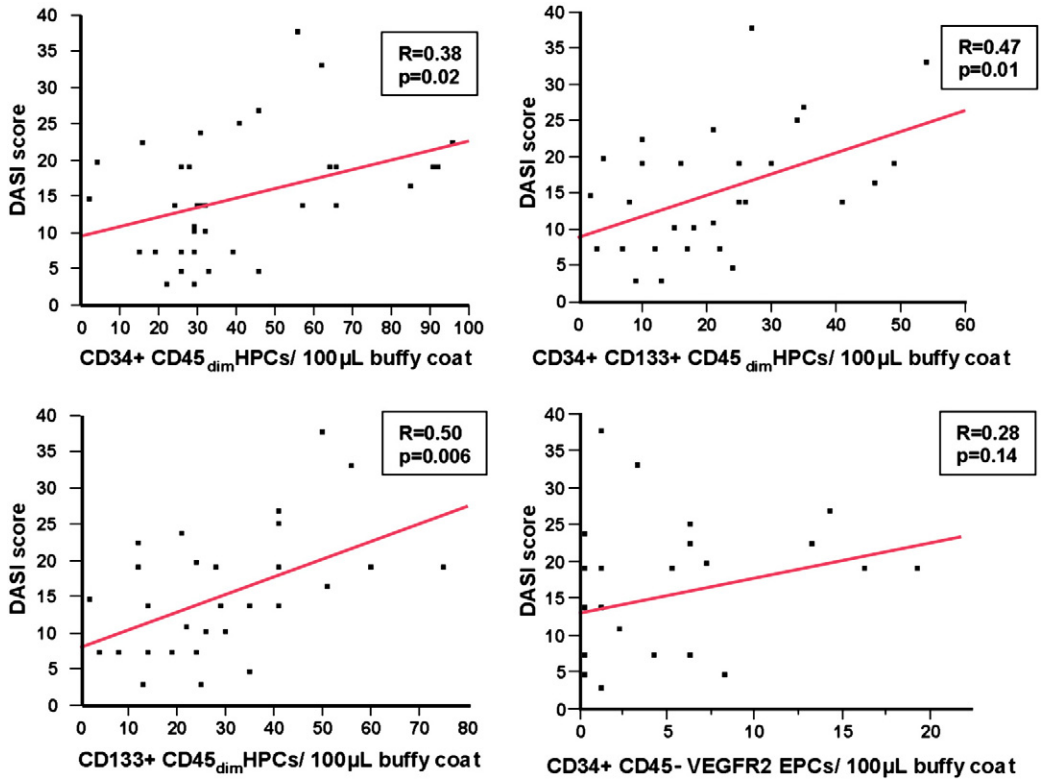

Fig. 3. Bivariate analysis of CPC counts and DASI scores. Correlations between CD34+, CD133+ and CD34+, CD133+ circulating progenitor cells are shown between DASI scores for all 13 patients at all time points. Correlations for the derived VO2 and METs estimates were identical to that for each cell type with the DASI score.

HPC counts also correlated with DASI scores in these patients, which also improved significantly from baseline and from which estimates of METs and peak VO2 may be derived [15]. This was not the case with cells fitting the description of the putative EPC (CD45-).

The restriction of this association to circulating haematopoeitic progenitor cell (HPC) is intriguing as these $CD45_{dim}$ cells do not form endothelial cells in culture—they are distinctly haematopoietic in nature [13,14]. Previous evidence has demonstrated the production of numerous growth factors including angiopoietins and VEGF by HPCs in vitro, growth factors integral in angiogenesis [26–28]. Co-culture experiments have shown enhanced in vitro angioblast cell growth in the presence of CD34+ haematopoietic progenitor cells [29]. In addition, there is a key interplay between endothelial cells and hematopoietic progenitors in the bone marrow [30–32]. It is therefore likely that circulating hematopoietic progenitors play a key role in endogenous vascular and cardiac repairs. Their depletion in numbers could conceivably result in downstream deleterious effects on endogenous self-renewal in cardiovascular disease. The paracrine effects of HPCs on resident endothelial cells and progenitors via production of angiopoietins and other cytokines may represent a potential mechanism for the relationship between circulating progenitor cells of haematopoietic lineage and observed cardioprotective effects [27].

We therefore propose that EECP, through mechanisms as yet unclear, induces increased production of HPCs by the bone marrow or decreased senescence of HPCs in the circulation, with a resultant enhanced paracrine effect on resident progenitor cells in the vascular beds and promotion of endogenous self repair.

5. Study limitations

This was a single center study in a small cohort of patients without a preliminary sample size calculation which limits statistical power. However, complete follow-up was available in all patients in this prospective study and the findings of our study are indeed novel with respect to providing an insight into the mechanisms by which EECP achieves its effects.

6. Conclusions

This is the first prospective study of progenitor cells in vascular biology to suggest an association between functional measures of improvement post EECP therapy and circulating progenitor cell counts in patients with refractory angina pectoris. These findings are additive to our emerging understanding of the underlying mechanisms of the pro-angiogenic effects of EECP.

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Disclosures

None.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [33].

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