

Improvement of fractional flow reserve and collateral flow by treatment with external counterpulsation (Art.Net.-2 Trial)

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ABSTRACT

Background Arteriogenesis (collateral artery growth) is nature's most efficient rescue mechanism to overcome the fatal consequences of arterial occlusion or stenosis. The goal of this trial was to investigate the effect of external counterpulsation (ECP) on coronary collateral artery growth.

Materials and methods A total of 23 patients (age 61 ± 2.5 years) with stable coronary artery disease and at least one haemodynamic significant stenosis eligible for percutaneous coronary intervention were prospectively recruited into the two study groups in a 2 : 1 manner (ECP : control). One group (ECP group, $n = 16$) underwent 35 1-h sessions of ECP in 7 weeks. In the control group ($n = 7$), the natural course of collateral circulation over 7 weeks was evaluated. All patients underwent a cardiac catheterization at baseline and after 7 weeks, with invasive measurements of the pressure-derived collateral flow index (CFI_p, primary endpoint) and fractional flow reserve (FFR).

Results In the ECP group, the CFI_p (from 0.08 ± 0.01 to 0.15 ± 0.02 ; $P < 0.001$) and FFR (from 0.68 ± 0.03 to 0.79 ± 0.03 ; $P = 0.001$) improved significantly, while in the control group no change was observed. Only the ECP group showed a reduction of the Canadian Cardiovascular Society (CCS, $P = 0.008$) and New York Heart Association (NYHA, $P < 0.001$) classification.

Conclusion In this study, we provide direct functional evidence for the stimulation of coronary arteriogenesis via ECP in patients with stable coronary artery disease. These data might open a novel noninvasive and preventive treatment avenue for patients with non-acute vascular stenotic disease.

Keywords Arteriogenesis, collateral circulation, coronary artery disease, external counterpulsation.

Eur J Clin Invest 2009

Introduction

Coronary artery disease (CAD) remains a key burden to the industrialised world despite well-approved noninvasive (oral medication, healthy and active lifestyle) [1] as well as invasive therapeutic options. The latter involves angioplasty and/or

surgical bypass surgery. Both procedures are clinically safe, but not all patients are suitable for these therapies. Thus supplementary treatments as add-on strategies are in need to control symptoms and to alter the course of advanced CAD.

Arteriogenesis is nature's most efficient endogenous rescue mechanism to overcome the fatal consequences of vascular

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occlusion/stenosis [2]. The rapid proliferation of pre-existing collateral pathways has a significant impact on improving jeopardised distal tissue perfusion upon proximal artery occlusion or relevant stenosis. However, in several cases, the speed of arteriogenic proliferation may not be efficient enough to avoid ischaemic tissue damage. Thus, the therapeutic stimulation of arteriogenesis is an attractive noninvasive treatment option in subjects with vascular stenotic disease.

Arteriogenesis is triggered by biomechanical forces, in particular increased levels of shear stress across newly recruited collaterals. Increased blood flow resulting in enhanced endothelial shear stress within these arteriolar/arterial anastomoses activates the endothelium and initiates the process of vascular remodelling. If pressure gradients and thus shear stress levels remain high during this initial process of collateral recruitment, the conductance of the resulting vessels can reach or even surpass the conductance of the occluded artery [3]. Moreover, chemokine expression is enhanced and leads to adhesion/transmigration of pluripotent mononuclear cells (monocytes) as well as recruitment of resident macrophages, which orchestrate the remodelling of small collateral arterioles/arteries into large conductance arteries in a paracrine fashion [4].

The physiological impact of the human coronary collateral circulation is beyond doubt; well-developed collateral arteries reduce the size of myocardial infarction [5], and nonfatal as well as fatal cardiac events [6]. Recent clinical investigations provided evidence for the therapeutic induction of arteriogenesis via the biomechanical (shear stress) and/or the paracrine fashion:

Senti *et al.* [7] demonstrated that long-term physical activity and the severity of a coronary artery stenosis are independently and directly associated with sufficient collateral networks detected invasively via the flow-dependent collateral flow index. Zbinden *et al.* [8] showed that the rapid decline of coronary collateral flow after percutaneous coronary intervention (PCI) [9] can be prevented by exercise training; even in normal vessels, collateral flow was increased significantly upon endurance training. Pohl demonstrated that the severity of coronary artery stenosis is the most important predictor for a well-developed collateral circulation [10]. These data are in line with the experimental findings that increased shear stress across collaterals due to stenosis/occlusion or exercise training directly correlates with the conductance of collateral circulation.

Seiler and colleagues were the first to show that subcutaneous granulocyte-macrophage colony-stimulating factor (GM-CSF) leads to a significant increase of collateral flow in patients with CAD [11]; thus providing evidence for the paracrine strategy.

The objective of this clinical trial is based on reports about beneficial effect of ECP in patients suffering from CAD. ECP is a noninvasive technique augmenting diastolic blood flow

volume and blood pressure in the vascular bed and decreasing systolic pressure. This effect is achieved by three pairs of pneumatic cuffs wrapped around the lower extremities. The cuffs inflate, triggered by ECG, at the onset of diastole (diastolic augmentation, increased venous return); and deflate rapidly at the onset of systole (systolic unloading). The effect of ECP has been investigated in numerous trials. Clinical benefits including relief of angina symptoms, increased exercise tolerance and improved quality of life have been reported, whereas no clinical benefits have been observed in ECP-sham treated groups [12]. ECP results in a reduction of ischaemic defects and a sustained improvement of myocardial perfusion [13,14]. Suggested mechanisms contributing to the clinical benefit include improvement in endothelial function, recruitment of coronary collaterals and a decrease of peripheral vascular resistance [12].

However, to date no data exists on whether ECP promotes active growth of functional collateral arteries (arteriogenesis).

In this study we present, to the best of our knowledge, the first clinical proof-of-concept trial providing functional evidence for the role of ECP in the setting of clinical arteriogenesis. By using the gold standard invasive method to detect myocardial collateral dependent blood flow, the CFIP (pressure-derived collateral flow index), we demonstrate a direct therapeutic effect of ECP on coronary collaterals in patients with stable angina pectoris and significant coronary stenosis.

Methods

Patients

Patients with stable CAD were screened for study eligibility based on the following major criteria: (1) angiographical narrowing (>70%) of at least one coronary artery diagnosed by visual assessment within the last 2 months; (2) no previous infarction in the myocardial region of interest (ROI) for the assessment of collateral blood flow; (3) positive noninvasive ischaemic testing for the ROI; (4) no congestive heart failure; (5) no contraindication for adenosine or ECP [15] and (6) being able to take part in the study for 7 weeks, 5 days per week. Patients were eligible for inclusion as soon as haemodynamic significance was verified during the catheterization (pressure-derived Fractional Flow reserve <0.8 and positive noninvasive ischaemic imaging).

The study was conducted in accordance with the principles of the declaration of Helsinki and approved by the Ethical Committee of the Charité in 09/2006. All patients gave written informed consent to participate in the study before enrolment.

Cardiac catheterization procedure

The cardiac catheterization procedure was performed on a standard angiography suite (Hicor, Siemens). Catheters without side holes were used. Weight adjusted heparin was adminis-

tered intravenously. Left ventricular end-diastolic pressure was determined at the beginning. 0.2 mg nitroglycerine was administered intracoronarily into the vessel of interest and repeated every 20 min throughout the procedure. Biplane coronary diagnostic angiography was performed routinely and coronary artery stenosis was quantitatively assessed (QCA) offline. If the stenosis of interest was significant and of low risk [16], the procedure was continued with pressure measurements.

Haemodynamic measurements

An interval of 10 min was allowed for dissipation of the effect of nonionic contrast agent on coronary vasomotion. Pressure-derived fractional flow reserve (FFR) served in this study protocol as an inclusion criterion as well as a secondary endpoint. Only one lesion of a major coronary artery was assessed by FFR in each patient. No bypass graft was assessed. The mean aortic pressure (P_a mmHg) was measured via the guiding catheter; mean central venous pressure (P_v mmHg) was measured with a catheter placed in the right atrium. Mean distal coronary pressure (P_d mmHg) and transit mean times (T_{mn}) were obtained using a 0.014" guidewire (PressureWire[®] 5 Radi Medical Systems) positioned distal to the stenosis. For assessment of FFR and the index of microcirculatory resistance (IMR), steady state hyperaemia was achieved with systemic application of adenosine ($140 \mu\text{g kg}^{-1}\text{min}^{-1}$) through a large antecubital vein. FFR was calculated ($\text{FFR} = P_d - P_v / P_a - P_v$) [17] and 3 consecutive thermodilution curves were obtained by brisk injection of 3 mL of room temperature saline into the coronary artery. Data were displayed in real-time (RadiAnalyzer[®] Express). IMR was calculated offline as described below [18]. If FFR was ≥ 0.8 , the patient was not eligible for inclusion. Further therapeutic procedures were performed independently of the study protocol. If FFR was < 0.8 and a positive noninvasive myocardial ischaemic imaging was existent for the ROI [19], the patient was included. The protocol was continued with assessment of the pressure-derived collateral flow index (CFI_p), the primary endpoint of the study. For coronary collateral assessment, an adequately sized balloon was placed right proximal to the stenosis in the non-stenotic segment. For measurement of the coronary wedge pressure (P_w mmHg), the balloon was inflated at low pressure (1–3 atm) until the antegrade coronary flow was interrupted. The transient complete occlusion was controlled via the real-time pressure measurement display and by injection of contrast dye. CFI_p was determined at the end of a 60-s occlusion described and calculated as $(P_w - P_v) / (P_a - P_v)$ [20]. The IMR was calculated offline as $(\text{IMR} = P_a * T_{mn} * (P_d - P_w / P_a - P_w))$ by taking the coronary wedge pressure into account as indicated in the presence of a significant stenosis [18]. The position of the pressure-wire distal to the stenosis was carefully documented in biplane angiography for the invasive follow-up.

Exercise test

A symptom-limited bicycle ergometric test (Kiss/eBikeL) was performed at baseline the day before the invasive procedure and with an identical protocol at the 8th week. The test started at 25 or 50 Watt and continued with a raise of 25 Watt every 2 min. The examination was terminated according to the guidelines [21]. The achievable workload, rate-pressure product, exercise duration, maximal heart rate and time to appearance of electrocardiographic changes were recorded.

Study protocol

At the time of the ethics approval in 09/2006, no permission for inclusion of a sham ECP or a control group was obtained from the committee. Hence the study was started with the recruitment of the ECP group. However, with the publication of the COURAGE Trial [1], providing evidence that under optimal medical treatment PCI can be deferred safely in stable angina pectoris, we received a positive ethical vote for the inclusion of a prospective control group. Three patients had already been recruited in the ECP group before the final ethical approval. Thereafter all included study participants were pseudo-randomised in a 2 : 1 manner to ECP and control. As already three patients were in the ECP group, the 4th included patient was allocated to the control group and from there on every third consecutive patient was also allocated to the control. To assess symptoms, standardised questionnaires on the Cardiovascular Society (CCS) and the New York Heart Association (NYHA) functional class were used. Within 3 weeks prior to the invasive diagnostics, oral antihypertensive medication was adjusted to meet the guideline recommendations [22]. A positive recent noninvasive ischaemic test for the area supplied by the vessel of interest (Table 1) was available for all patients.

All patients had an echocardiography at baseline. Throughout the study, patients were instructed not to change their daily activity. Patients were eligible for inclusion as soon as haemodynamic significance via FFR was proven. Thereafter, baseline medication remained unchanged throughout the study. The ECP group received 35 h of treatment in 7 weeks (5 h/week) with an EEC-PS3 device (Vasomedical Inc., Westbury, NY, USA). Clinical symptoms, status and the diastolic-to-systolic ratio (D/S) were registered at each treatment session. ECP was performed to achieve a target D/S ratio > 1.0 . To compensate for the non-therapy related effect of ECP, the control group had over 7 weeks, 5 times/week, walk-in appointment (for non-study related diagnostics, nutrition-counselling and physician's appointment). In the 8th week the invasive follow-up was performed. After having assessed the study-related haemodynamic measurements, revascularization was either performed or not performed according to the guidelines [19]. To prevent bias, invasive data were analysed by an experi-

Table 1 Baseline data

Category	Subcategory	ECP (n = 16)	Control (n = 7)	P-value
Demographic characteristics	Age, years (range)	62.3 (43–77)	61.4 (41–75)	0.87
	Male gender, n (%)	11 (68.8)	6 (85.7)	0.62
	BMI (kg m ⁻²)	28.5 ± 1.03	28.1 ± 1.25	0.84
Cardiovascular risk factors	Hypertension, n (%)	16 (100)	7 (100)	–
	Hyperlipaemia, n (%)	15 (93.8)	7 (100)	1.0
	Diabetes, n (%)	2 (12.5)	2 (28.5)	0.55
	Family history for CAD, n (%)	4 (25)	2 (28.6)	1.0
	Ongoing smoking, n (%)	3 (18.8)	1 (14.3)	1.0
Cardiac history and current status	Prior myocardial infarct, n (%)	8 (50)	3 (42.9)	1.0
	Prior percutaneous intervention, n (%)	13 (81.3)	6 (85.7)	1.0
	Prior CABG, n (%)	1 (6.3)	0 (0)	1.0
	Ejection fraction, %	62 ± 1.72	58 ± 1.25	0.11
	Systolic blood pressure (mmHg)	122 ± 2.81	120 ± 7.15	0.77
	Diastolic blood pressure (mmHg)	70 ± 1.76	72 ± 3.4	0.69
	Angina pectoris CCS class 0/I/II/III (%)	56/13/25/6	43/14/29/14	0.91
	NYHA Class I/II/III (%)	25/63/12	43/43/14	0.82
	Active way of life, n (%)	8 (50)	4 (57.1)	1.0
Baseline stress test	SPECT/CMR/stress-echocardiography	3/11/2	2/4/1	–
Blood results	Total cholesterol (mg dL ⁻¹)	173 ± 15.7	162 ± 12.2	0.65
	LDL(mg dL ⁻¹)	101 ± 11.3	90 ± 12.5	0.53
	HDL(mg dL ⁻¹)	47.4 ± 4	49.4 ± 9.8	0.82
	CRP (mg dL ⁻¹)	2.2 ± 0.86	1.43 ± 0.72	0.69

BMI, body-mass index; CABG, coronary artery bypass graft; CCS, Canadian Cardiovascular Society; CMR, cardiac magnetic resonance; CRP, C-reactive protein; ECP, external counterpulsation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NYHA, New York Heart Association; SPECT, Single Photon Emission Computed Tomography.

enced cardiologist (Klauss V., Munich) blinded to the study protocol; noninvasive data were analysed by blinded physicians (Pagonas N, Gross M).

Sample size and statistical methods

Evidence for ECP-related improvement of myocardial blood flow due to collateral arterial growth is provided by former PET studies [14,23]. PET is considered as a reliable method to detect collaterals noninvasively. The methodological error of CFIP and FFR measurement was taken into account [11,17]. Power calculations are based on the assumptions: One-tailed test for increase in myocardial blood flow in the ROI; significance level of 5%; power to detect change 80%. A mean change of myocardial blood flow respectively collateral blood flow of plus 15% in the ROI.

For the ECP group, a sample size of 12 was required. Taking a drop-out rate of 20–30% into account, 16 patients were planned to be included. Based on published data on the natural course of the collateral circulation (CFIP) wherein a reduced variance is observed, [6,8] a 2 : 1 ratio for the control group was planned, resulting in a sample size of 7 (6 + 1 expected drop-out). Intra-individual comparisons of baseline/follow-up data were performed using the paired Student t-test and Wilcoxon test. Between-group comparisons were performed by t-test and ANOVA or by Mann–Whitney test and Friedman test. A chi² test using Fisher's exact test was applied for comparison of categorical variables among the study groups. Linear regression analysis was performed to assess the association between D/S ratio and the invasive endpoints. Mean values ± SE are given. Statistical significance was defined at *P* < 0.05. All analyses

were calculated with SPSS-15.0. All statistical analyses were performed by a professional statistician.

Results

Baseline characteristics

A total of 33 patients met all the screening criteria and were assessed invasively. Ten of 33 patients did not meet the final inclusion criteria (FFR ≥ 0.8 or the pressure wire could not be placed in ROI). A total of 23 patients were finally enrolled in both groups (16/ECP, 7/control). Clinical baseline characteristics were well balanced in-between the groups (Table 1). There was no significant difference of the baseline CCS and NYHA classes between the groups. No difference of the medication was found between the groups. The haemodynamic severity of the assessed stenosis at baseline, as detected by FFR and QCA, did not differ between the groups. Baseline CFIP in the control group was higher compared with that of ECP (Table 2).

Invasive measurements

In the ECP group, the CFIP increased from 0.08 ± 0.01 to 0.15 ± 0.02 ($P < 0.001$), whereas the control group showed no variance of the index (0.15 ± 0.03 to 0.14 ± 0.02 , $p = 0.7$) demonstrating growth of collateral arteries in patients treated with ECP (Fig. 1). In accordance with the CFIP, the FFR index increased in the ECP group from 0.68 ± 0.03 to 0.79 ± 0.03 ($P = 0.001$) but not in the control group (0.68 ± 0.06 to 0.70 ± 0.05 , $P = 0.4$, Fig. 2). A total of 2/16 patients in the ECP group and 1/7 in the control group had to be excluded from analysis after completion of the trial due to protocol violation (ECP) and an arteriovenous-fistula in the ROI (control);

however, the number needed to treat ($n = 12$ and $n = 6$) was maintained. The treatment related effect of ECP remained significant albeit all data (plus excluded patients) were analysed ($n = 16$, $P = 0.018$ and $P = 0.004$ for the change of CFIP and FFR, respectively).

In both groups, no difference of the invasive endpoints in relation to other baseline factors was found (Table 3).

Secondary noninvasive clinical endpoints

In the ECP group, after treatment a significant reduction of the CCS classification was achieved ($P = 0.008$), whereas in control group no change ($P = 0.25$) was observed. The severity of dyspnoea (NYHA scale) was reduced after ECP ($P < 0.001$) but not within the control ($P = 0.28$). At the end of the therapy, 81% of the ECP patients were free of angina pectoris (CCS = 0) compared to 56% at baseline. In the control group, at baseline and after 7 weeks only one patient (14.3%) was free of angina. No patient treated with ECP had an increase in angina class or remained in CCS $> II$ after the therapy.

For the exercise test, there was statistically no significant difference in any of the evaluated parameters. The ECP group tended to achieve a higher exercise level after ECP (from 110 ± 5.88 to 117 ± 6.76 Watt, $P = 0.08$, NS).

D/S ratio

The D/S ratio had a significant improvement throughout the therapy. Mean D/S ratio increased from 0.86 ± 0.06 at baseline to 1.07 ± 0.08 in the 7th week ($P < 0.0001$). The improvement of FFR correlated significantly with the improvement of the D/S ratio ($P = 0.001$, $r = 0.54$). Between $\Delta D/S$ ratio and $\Delta CFIP$, a trend for a correlation was seen ($P = 0.06$).

Table 2 Baseline angiographic and haemodynamic data

Category	ECP	Control	P-value
One-/two-/three-vessel disease, n	6/6/2	1/2/3	0.33
Vessel LAD/LCX/RCA, n	8/2/4	5/0/1	0.63
Diameter stenosis of ROI, (%)	52.4 ± 3.46	54.2 ± 4.3	0.76
Fractional flow reserve, no unit	0.68 ± 0.03	0.68 ± 0.05	0.89
Left ventricular end-diastolic pressure (mmHg)	11.6 ± 0.72	13.5 ± 1.5	0.21
Mean aortic pressure at occlusion (P_a), mmHg	89.8 ± 3.86	91.3 ± 3.61	0.82
Coronary occlusive pressure (P_w), mmHg	10.2 ± 1.38	17 ± 2.77	0.025*
CVP during occlusion, mmHg	3.79 ± 0.59	5.25 ± 1.56	0.29
Collateral flow index, no unit	0.08 ± 0.01	0.15 ± 0.03	0.016*

CVP, central venous pressure; ECP, external counterpulsation; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; ROI, region of interest.

* $P < 0.05$.

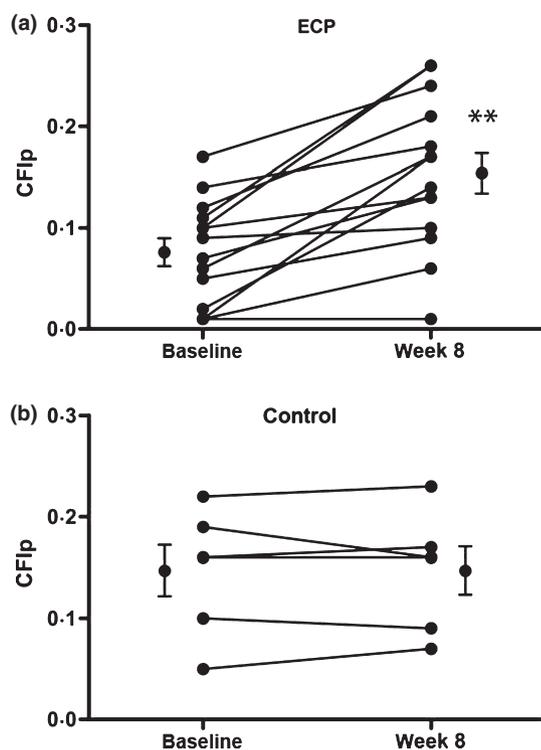


Figure 1 Changes of CFIP from baseline to week 8. CFIP, pressure-derived collateral flow index; ECP, external counterpulsation.

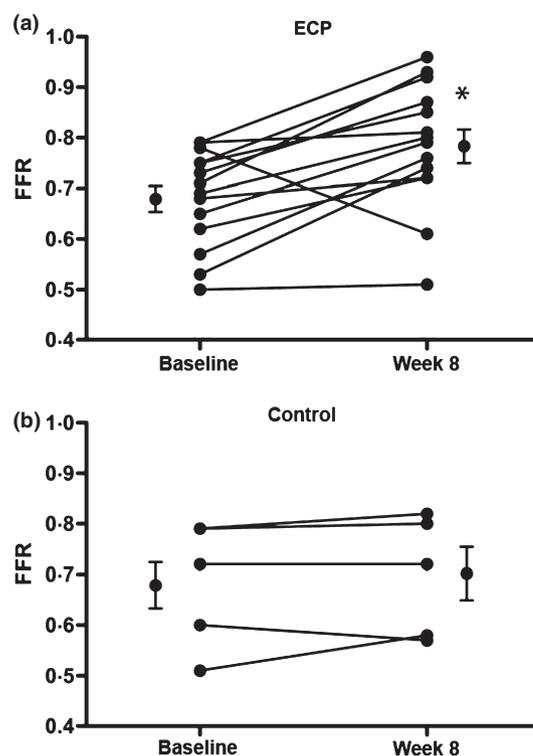


Figure 2 Changes of FFR from baseline to week 8. FFR, fractional flow reserve, ECP, external counterpulsation.

Adverse events

No major adverse event occurred during the study. Minor adverse events such as sore muscles after the therapy were observed in the ECP group. No special therapy was necessary.

Discussion

Important hallmarks of arteriogenesis are enhanced levels of shear stress across recruited collateral pathways. Experimentally, a therapeutic increase in shear stress may be achieved via artificial stenosis, ligation or arterio-venous shunting distal to the site of occlusion/stenosis [3]. ECP increases arterial shear stress even in the absence of a stenosis [24]. In humans, it is proposed as an elegant method to enhance shear stress noninvasively in the arterial system (within the cardiac diastole without increasing heart rate) [12,25]. In this proof-of-concept trial, we evaluated whether counterpulsation leads to a significant change in CFIP and FFR as compared with the natural time course under optimal medical treatment.

The primary hypothesis of the Art.Net.-2 Trial was to investigate the direct effects of counterpulsation on collateral function in patients with stable angina pectoris using the CFIP. The latter

is currently the gold standard for the assessment of the collateral circulation. CFIP expresses the maintained collateral blood flow during coronary occlusion relative to normal antegrade flow during vessel patency [20]. In contrast to several other trials – which detected CFIP either at single time points or performed CFIP measurement within the PCI procedure – the Art.Net.-2-Trial assessed CFIP at two longitudinal time points: baseline and follow-up after 7 weeks. Patients with severe coronary artery stenosis, known to be at higher risk of periprocedural complications [16], were excluded due to ethical concerns. Consequently, the mean residual coronary artery stenosis (assessed by QCA) of patients in our trial was $53 \pm 2.63\%$.

We observed a significant increase of collateral blood flow after ECP treatment, whereas in the control group no change was observed. The intra-individual CFIP changes within the ECP group were heterogeneous. We identified 12/14 CFIP responders (improvement of CFIP) and 2/14 CFIP non-responders (no improvement of CFIP). Lower pretreatment fractional flow reserves, hence more relevant stenoses were associated with responders (9/12 of CFIP responders had FFR at baseline <0.75). The significant – however moderate – increase of CFIP (0.07 ± 0.016) in our study is explained with the heterogeneity of patients with moderately severe or severe

Table 3 Angiographic and haemodynamic results

Category	ECP			Control		
	Baseline	Week 8	P-value	Baseline	Week 8	P-value
Systolic BP (mmHg)	121.9 ± 2.81	120.4 ± 2.87	0.53	120 ± 7.15	124 ± 3.41	0.52
Diastolic BP (mmHg)	70.4 ± 1.76	69.7 ± 1.85	0.45	72 ± 3.44	74 ± 3.08	0.22
CFI _p , no unit	0.08 ± 0.01	0.15 ± 0.02	<0.001*	0.15 ± 0.03	0.14 ± 0.02	0.67
FFR, no unit	0.68 ± 0.03	0.79 ± 0.03	0.001†	0.68 ± 0.05	0.70 ± 0.05	0.39
IMR, no unit	14.2 ± 2.47	15.2 ± 1.43	0.76	10.1 ± 2.48	12 ± 0.86	0.49
T _{mn} (s)	0.28 ± 0.05	0.28 ± 0.04	0.98	0.21 ± 0.04	0.34 ± 0.08	0.25
Pa (mmHg)	80.9 ± 4.1	81.1 ± 4.8	0.96	80.6 ± 3.2	79 ± 9.4	0.87
Pd (mmHg)	55.4 ± 2.4	63.1 ± 3.8	0.049†	55.8 ± 4.9	56.8 ± 9.3	0.9
Pw (mmHg)	10.2 ± 1.4	18.6 ± 2.3	<0.001*	17 ± 2.8	19.2 ± 2.7	0.31
LVEDP (mmHg)	11.6 ± 0.72	11 ± 0.54	0.24	13.5 ± 1.5	12.5 ± 1.18	0.31
Diameter stenosis (%)	52.4 ± 3.46	49.9 ± 4.42	0.22	54.2 ± 4.3	55.0 ± 4.66	0.29

BP, blood pressure; CFI_p, pressure-derived collateral flow index; ECP, external counterpulsation; FFR, fractional flow reserve; IMR, index of microvascular resistance; LVEDP, left ventricular end-diastolic pressure; T_{mn}, hyperaemic transit mean time; Pa, aortic pressure under maximal vasodilatation; Pd, distal coronary pressure under maximal vasodilatation; Pw, coronary occlusive pressure.

*P < 0.001, †P < 0.05.

stenosis. The close correlation of the degree of stenosis and the conductance of collaterals (CFI_p) is in accordance with earlier experimental data in this field by Schaper [26] as well as several clinical trials [6,10].

Although functional severity of the stenosis (Table 3) and symptoms at baseline (Table 1) were well matched, collateral blood flow differed in between the groups, reflecting the high inter-individual range of the collateral status. These inter-individual variations are well known in CAD patients – as previously published, [6,8,10,11] and provoked in patients with poor collateralization (CFI_p <0.25). In our trial, all patients had an insufficient collateralization at baseline and a significant increase in collateral blood flow only after ECP treatment.

Recently, Seiler investigated the effect of GM-CSF on the collateral circulation by sequential CFI_p measurements [11]; the degree of stenosis at baseline was more severe and concomitantly the mean CFI_p at baseline (0.21 ± 0.14) was higher. After therapeutic delivery of GM-CSF over 2 weeks, collateral conductance increased significantly but also with a wide range within the response (0.11 ± 0.12) compared to our study (0.07 ± 0.016). However, due to the differing degree of stenosis and the time-course (2 weeks GM-CSF vs. 7 weeks ECP), no inference can be drawn in terms of the effectiveness of each treatment. Our data indicate that external counterpulsation treatment, even in a rather heterogenous group with moderate and moderate-severe grade of stenosis, leads to a significant functional improvement of the collateral circulation.

The FFR is the most reliable tool in the catheterization laboratory to assess the physiological relevance of a stenosis [17]. Supposing that myocardial resistance is minimal under adenosine, the ratio of distal coronary to aortic pressure reflects regional myocardial blood flow arising from epicardial, intramyocardial and subendocardial coronary arteries and collaterals. An improvement of the FFR, due to the distal coronary pressure in particular, mirrors an improvement of blood flow to the myocardium distal to the stenosis. As FFR reflects the limitation of myocardial perfusion due to the epicardial resistance, FFR is directly depending on the morphology of stenosis. Further, an influence of the post-stenotic microcirculation cannot be completely ruled out. Regarding this, QCA and the resistance of the myocardial microcirculation were assessed at baseline and at follow-up.

In the ECP group, the FFR increased significantly (Table 3), whereas in the control no change was observed. Within the ECP group, we identified 13/14 FFR responders (improvement of FFR) and 1/14 FFR non-responder (decline of FFR). The improvement of myocardial blood flow at demand (vasodilatation) may be strengthened by the fact that the angiographically recorded stenosis narrowing (QCA % diameter of stenosis) and the resistance of the microcirculation remained unchanged in the ECP group as well as in the control group (Table 3). Hence, the improvement of FFR reflects the 'true' improvement of myocardial blood flow and is not based on changes in the degree of stenosis or microvascular resistance [27].

All study participants were qualified for PCI at baseline [19]. At the end of the therapy course, 6/16 patients in the ECP group vs. 1/7 patients in the control group were deferred from angioplasty. In summary, the significant increase in CFIp is supported by a second functional endpoint, the FFR. Thus pressure-derived myocardial blood flow reserve improved upon ECP treatment significantly, reflecting the improvement of myocardial blood flow in the area at risk.

The status of the coronary microcirculation is of clinical and prognostic relevance. Recently a novel parameter, the index of IMR, was introduced to assess the microvascular resistances [28]. IMR seems also to be a reliable index to assess the microvascular status in the presence of a significant stenosis. This is achieved by taking the contributing collateral blood flow into account. An IMR 22 (no units) was observed in patients with stable CAD and without obvious microvascular dysfunction, and is proposed as a reference for an undisturbed microcirculation [18]. In our study, the mean IMR 12.9 at baseline suggests that most of the patients may have a normal microvascular function. After ECP treatment, as well as in the control group, no significant change of the IMR was observed; taking IMR <22 as reference, the resistance of the microcirculation remained within a normal range. Only one patient in the ECP group had a pathological IMR (40.5) at baseline, which improved clearly at the end of the therapy (IMR 9).

Regarding the noninvasive endpoints of the trial, a significant reduction of angina (CCS-Score) and dyspnoea at exercise (NYHA) was demonstrated after the ECP therapy. These results are in accordance with previous data [29]. ECP led either to complete relief from angina or to an increase of symptoms threshold.

In the current trial, the reduction in angina seen in all of the pre-ECP symptomatic patients was related to an improvement of the coronary functional status, suggesting that the amelioration of angina is considerably depending on the improvement of myocardial blood flow. However, due to the small number of symptomatic patients, these data have to be further investigated in future trials.

As all patients, including those who were free of angina at baseline, had an improvement of effort-related dyspnoea, the latter beneficial effect of ECP is likely to depend on synergistic effects, including improved diastolic function, increased exercise capacity and decreased peripheral vasculature resistance [12,30,31].

It is known that the growth of collateral arteries in humans, contrary to those in animals, is a process which takes place during weeks to months [32]. In this trial, the significant decrease of CCS and NYHA class was obtained in the second half of the therapy, suggesting that the commonly used ECP protocol of about >30 h of therapies is appropriate for an adequate clinical response.

The D/S ratio reflects the haemodynamic effect of ECP. According to the current knowledge [12], the improvement of the D/S ratio mirrors a decrease of the systolic pressure due to improved arterial stiffness deriving from ameliorated endothelial dysfunction. Within this trial, a significant positive correlation was found between Δ D/S ratio improvement and improvement of FFR; between Δ D/S and Δ CFIp no significant correlation, but a trend, was observed. This data is in line with numerous studies reporting that regular exercise results in 1) improved vascular reactivity related to ameliorated systemic endothelial function [33] as well as 2) enhanced myocardial perfusion, mostly due to adaptive collateral artery growth [7,8,34]. As 60% (ECP group) and 50% of patients (control) were limited due to orthopaedic problems, treadmill testing was not suitable as a clinical endpoint.

The protective and beneficial effect of well-developed myocardial collateral circulation on myocardial tissue as well as survival in subjects with vascular occlusive disease was demonstrated independently by several investigators [2,6]. Thus, the impact of well-developed collateral arteries is beyond doubt and the therapeutic stimulation of the latter remains to be an attractive clinical goal.

Some limitations of this study are a) the lack of a sham group and b) the non-randomised design of the trial: In terms of a) the local Ethical Committee did not approve to treat patients with sham treatment, due to the invasive endpoints. Sham ECP treatment (low pressure <80 mmHg) vs. active ECP treatment (low pressure >220 mmHg) had been applied in previous clinical trials, and therapeutic effects were observed only in the groups that had received active ECP treatment. To compensate for this lack of a sham group, we applied for a control group receiving optimal medication and counselling, as performed in COURAGE. The positive vote for this control was obtained after the trial was initiated; hence, a 'regular' randomization with the start of the trial was no longer feasible.

In conclusion, we present in this study the first data about noninvasive stimulation of arteriogenesis via ECP in patients with stable angina pectoris. Based on diastolic augmentation and consequently enhanced shear forces, this concept of 'passive coronary training' may serve as a valuable complementary treatment strategy to promote myocardial collateral growth in patients beyond active training as well as experimental cytokine or cell therapies. It is reasonable to speculate that patients, especially those not being capable to perform regular cardiovascular exercise, might profit from such 'passive collateral coronary training'. Further studies are under way to prove the benefit of this noninvasive pro-arteriogenic form of therapy.

Conflict of interest

None declared.

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Received 2 May 2009; accepted 5 June 2009

References

- Boden WE, O'Rourke RA, Teo KK, Hartigan PM, Maron DJ, Kostuk WJ *et al.* Optimal medical therapy with or without PCI for stable coronary disease. *N Engl J Med* 2007;**356**:1503–16.
- Werner GS, Ferrari M, Betge S, Gastmann O, Richartz BM, Figulla HR. Collateral function in chronic total coronary occlusions is related to regional myocardial function and duration of occlusion. *Circulation* 2001;**104**:2784–90.
- Eitenmuller I, Volger O, Kluge A, Troidl K, Barancik M, Cai WJ *et al.* The range of adaptation by collateral vessels after femoral artery occlusion. *Circ Res* 2006;**99**:656–62.
- Heil M, Schaper W. Influence of mechanical, cellular, and molecular factors on collateral artery growth (arteriogenesis). *Circ Res* 2004;**95**:449–58.
- Sabia PJ, Powers ER, Ragosta M, Sarembock IJ, Burwell LR, Kaul S. An association between collateral blood flow and myocardial viability in patients with recent myocardial infarction. *N Engl J Med* 1992;**327**:1825–31.
- Meier P, Gloekler S, Zbinden R, Beckh S, de Marchi SF, Zbinden S *et al.* Beneficial effect of recruitable collaterals: a 10-year follow-up study in patients with stable coronary artery disease undergoing quantitative collateral measurements. *Circulation* 2007;**116**:975–83.
- Senti S, Fleisch M, Billinger M, Meier B, Seiler C. Long-term physical exercise and quantitatively assessed human coronary collateral circulation. *J Am Coll Cardiol* 1998;**32**:49–56.
- Zbinden R, Zbinden S, Meier P, Hutter D, Billinger M, Wahl A *et al.* Coronary collateral flow in response to endurance exercise training. *Eur J Cardiovasc Prev Rehabil* 2007;**14**:250–7.
- Zimarino M, Ausiello A, Contegiacomo G, Riccardi I, Renda G, Di Iorio C *et al.* Rapid decline of collateral circulation increases susceptibility to myocardial ischemia: the trade-off of successful percutaneous recanalization of chronic total occlusions. *J Am Coll Cardiol* 2006;**48**:59–65.
- Pohl T, Seiler C, Billinger M, Herren E, Wustmann K, Mehta H *et al.* Frequency distribution of collateral flow and factors influencing collateral channel development. Functional collateral channel measurement in 450 patients with coronary artery disease. *J Am Coll Cardiol* 2001;**38**:1872–8.
- Seiler C, Pohl T, Wustmann K, Hutter D, Nicolet PA, Windecker S *et al.* Promotion of collateral growth by granulocyte-macrophage colony-stimulating factor in patients with coronary artery disease: a randomized, double-blind, placebo-controlled study. *Circulation* 2001;**104**:2012–7.
- Bonetti PO, Holmes DR Jr, Lerman A, Barsness GW. Enhanced external counterpulsation for ischemic heart disease: what's behind the curtain? *J Am Coll Cardiol* 2003;**41**:1918–25.
- Urano H, Ikeda H, Ueno T, Matsumoto T, Murohara T, Imaizumi T. Enhanced external counterpulsation improves exercise tolerance, reduces exercise-induced myocardial ischemia and improves left ventricular diastolic filling in patients with coronary artery disease. *J Am Coll Cardiol* 2001;**37**:93–9.
- Masuda D, Nohara R, Hirai T, Kataoka K, Chen LG, Hosokawa R *et al.* 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.
- Michaels AD, McCullough PA, Soran OZ, Lawson WE, Barsness GW, Henry TD *et al.* Primer: practical approach to the selection of patients for and application of EEC. *Nat Clin Pract Cardiovasc Med* 2006;**3**:623–32.
- Ryan TJ, Bauman WB, Kennedy JW, Kereiakes DJ, King SB III, McCallister BD *et al.* Guidelines for percutaneous transluminal coronary angioplasty. A report of the American Heart Association/American College of Cardiology Task Force on Assessment of Diagnostic and Therapeutic Cardiovascular Procedures (Committee on Percutaneous Transluminal Coronary Angioplasty). *Circulation* 1993;**88**:2987–3007.
- Pijls NH, De Bruyne B, Peels K, Van Der Voort PH, Bonnier HJ, Bartunek JKJ *et al.* Measurement of fractional flow reserve to assess the functional severity of coronary-artery stenoses. *N Engl J Med* 1996;**334**:1703–8.
- Aarnoudse W, Fearon WF, Manoharan G, Geven M, van de Vosse F, Rutten M *et al.* Epicardial stenosis severity does not affect minimal microcirculatory resistance. *Circulation* 2004;**110**:2137–42.
- Silber S, Albertsson P, Aviles FF, Camici PG, Colombo A, Hamm C *et al.* Guidelines for percutaneous coronary interventions. The Task Force for Percutaneous Coronary Interventions of the European Society of Cardiology. *Eur Heart J* 2005;**26**:804–47.
- Seiler C, Fleisch M, Garachemani A, Meier B. Coronary collateral quantitation in patients with coronary artery disease using intravascular flow velocity or pressure measurements. *J Am Coll Cardiol* 1998;**32**:1272–9.
- Trappe HJ, Lollgen H. Guidelines for ergometry. German Society of Cardiology – heart and cardiovascular research. *Z Kardiol* 2000;**89**:821–31.
- Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS *et al.* ACC/AHA 2002 guideline update for the management of patients with chronic stable angina—summary article: a report of

- the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on the Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 2003;**41**:159–68.
- 23 Masuda D, Fujita M, Nohara R, Matsumori A, Sasayama S. Improvement of oxygen metabolism in ischemic myocardium as a result of enhanced external counterpulsation with heparin pretreatment for patients with stable angina. *Heart Vessels* 2004;**19**:59–62.
- 24 Zhang Y, He X, Chen X, Ma H, Liu D, Luo J *et al.* Enhanced external counterpulsation inhibits intimal hyperplasia by modifying shear stress responsive gene expression in hypercholesterolemic pigs. *Circulation* 2007;**116**:526–34.
- 25 Vita JA, Mitchell GF. Effects of shear stress and flow pulsatility on endothelial function: insights gleaned from external counterpulsation therapy. *J Am Coll Cardiol* 2003;**42**:2096–8.
- 26 Schaper W. *The collateral circulation of the heart*. New York: American Elsevier Publishing Company, 1971.
- 27 Nico HJ, Pijls BdB. *Coronary pressure, Chapter 12.5.2-12.5.5*. Dordrecht: Kluwer Academic Publishers, 2000, page 251.
- 28 Fearon WF, Balsam LB, Farouque HM, Caffarelli AD, Robbins RC, Fitzgerald PJ *et al.* Novel index for invasively assessing the coronary microcirculation. *Circulation* 2003;**107**:3129–32.
- 29 Arora RR, Chou TM, Jain D, Fleishman B, Crawford L, McKiernan T *et al.* 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.
- 30 Levenson J, Simon A, Megnien JL, Chironi G, Gariépy J, Pernollet MG *et al.* Effects of enhanced external counterpulsation on carotid circulation in patients with coronary artery disease. *Cardiology* 2006;**108**:104–10.
- 31 Esmaeilzadeh M, Khaledifar A, Maleki M, Sadeghpour A, Samiei N, Moladoust H *et al.* Evaluation of left ventricular systolic and diastolic regional function after enhanced external counterpulsation therapy using strain rate imaging. *Eur J Echocardiogr* 2009;**10**:120–6.
- 32 Fulton WFM, Van Royen N. The coronary collateral circulation in man. In: Schaper W, Schaper J, editors. *Arteriogenesis*. Dordrecht: Kluwer Academic Publishers; 2004. pages 297–333.
- 33 Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S *et al.* Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med* 2000;**342**:454–60.
- 34 Bonetti F, Margonato A, Mailhac A, Carandente O, Cappelletti A, Ballarotto C *et al.* Coronary collaterals reduce the duration of exercise-induced ischemia by allowing a faster recovery. *Am Heart J* 1992;**124**:48–55.