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One-year follow-up of transcoronary sinus administration of autologous bone marrow in patients with chronic refractory angina

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Abstract

Purpose: Based on our preclinic studies with autologous unfractionated bone marrow (AUBM) via coronary sinus with transitory occlusion, a clinic study in patients with chronic refractory angina was designed. The objectives were to evaluate tolerance of the procedure, safety, and feasibility with 1 year follow-up.

Methods and materials: Clinical study with inclusion and exclusion criteria defined by an Independent Clinical Committee was carried out. Fifteen patients underwent transcoronary sinus administration with a 15-min occlusion of freshly aspirated and filtered AUBM (60–120 ml). Feasibility was evaluated with Seattle Angina Questionnaire (SAQ), Canadian Cardiovascular Society (CCS) angina classification, perfusion dipyridamole, and coronary angiography.

Results: There were no changes in the tolerance parameters. There were no deaths or myocardial infarction during the follow-up. Three patients were readmitted into the hospital. During the follow-up, one patient was diagnosed with cancer of the lung. Improvement of 30% in the quality of life was evaluated by SAQ. The CCS angina classification showed that the mean angina class was 3.0 ± 0.53 at baseline, which improved to 1.6 ± 0.63 at 1 year ($P < .001$).

Perfusion imaging (core lab) showed improvement in 12 of 15 patients, with a mean improvement of 40.9% at rest (22 vs. 13) ($P < .01$) and 45.3% at stress (26.5 vs. 14.5) ($P < .05$). Coronary angiography showed more collateral vessels in 10 of 15 patients.

Conclusions: We can conclude that AUBM via coronary sinus is feasible in patients with chronic refractory angina after 1 year follow-up, and it appears to be safe.

Keywords: Angiogenesis; Unfractionated bone marrow; Autologous bone marrow; Chronic refractory angina; Coronary sinus; No option patients; Collateral circulation

1. Introduction

Pharmacological treatments and revascularization procedures in patients with angina pectoris are meant to improve the ischemic threshold by reducing the myocar-

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dial oxygen demand or by improving the oxygen supply to the myocardium.

An increasing number of patients surviving various ischemic events are suffering from chronic refractory angina, refractory to conventional strategies with antianginal medication, percutaneous transluminal coronary angioplasty (PTCA) procedures, or coronary artery bypass graft (CABG) surgery.

This increasing clinical problem has stimulated interest in the use of angiogenesis factors to promote the growth of collateral blood vessels in ischemic tissues [1–4]. Given the complexity of the natural angiogenesis processes, the delivery of a single angiogenesis growth factor might provide a suboptimal stimulus to collateral development. Therefore, we tested a cell-based strategy based on the hypothesis that the cells secrete, in a time-and-concentration appropriate manner, multiple angiogenesis factors needed for optimal collateral development. In preclinical studies [5], we have demonstrated that administration of autologous unfractionated bone marrow (AUBM) to ischemic porcine myocardium enhanced angiogenesis, and that the coronary sinus as a route of administration was effective and well tolerated.

The present Phase I pilot study was designed to examine the safety, tolerance, and feasibility of transcoronary sinus administration of AUBM in patients with chronic refractory angina with 1 year follow-up. This investigation was approved by the institutional Ethics Committee, all procedures were reviewed by the Institutional Review Boards of the participating centers and were conducted in accordance with the Helsinki Declaration of 1975 (revised 1988), and all the patients gave written informed consent to participate in the study.

2. Methods

We used a multicenter prospective study in patients with chronic refractory angina. The patients were prospectively selected by an Independent Clinical Committee (ICC) on the basis of the following inclusion criteria: (1) symptomatic stable chronic angina (no significant changes in angina pattern for the least 6 weeks) despite the best medical treatment, (2) reversible perfusion defect on myocardial perfusion study, (3) coronary anatomy that was suboptimal for PTCA or CABG, (4) normal routine laboratory screening, and (5) willingness and ability to complete all components of the study and its follow-up. Patients were excluded if they had congestive heart failure or left ventricular ejection fraction <20%, myocardial infarction (<3 weeks), CABG (<6 months), PTCA (<6 months), new or unstable angina (<3 weeks), significant arrhythmias, severe valvular heart disease, restrictive or hypertrophic cardiomyopathy, hematopoietic disease, and conditions that may adversely affect bone marrow (BM) (such as malignancy or immunodeficiency).

Twenty-four patients with clinical history, nuclear myocardial perfusion study (dipyridamole), and cardiac catheterization were evaluated by the ICC, and 15 of them were eligible for the study. After they were included, the patients underwent clinical evaluation and completed a medical

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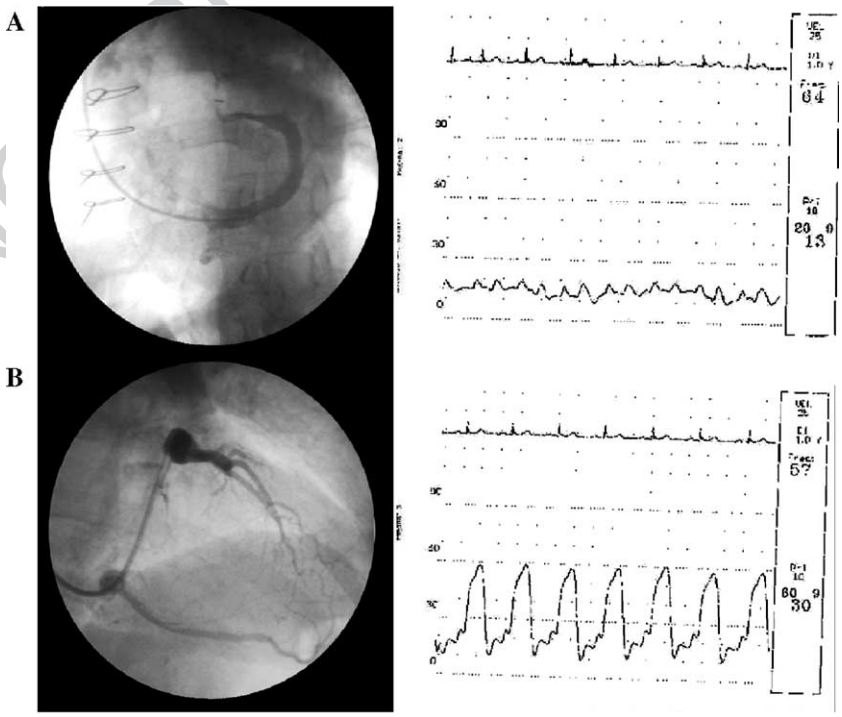

Fig. 1. Coronary sinus catheterization. (A) Venous angiography and pressure. (B) Venous angiography with coronary sinus occlusion and pressure.

t1.1 Table 1
t1.2 Baseline demographic and clinical data

t1.3 Characteristics	Mean±S.D. or *n* (%)
t1.4 Age (years)	65±9
t1.5 Male gender	12 (80)
t1.6 Angina Class III	11 (73)
t1.7 Angina Class IV	2 (13)
t1.8 Hypertension	13 (86.6)
t1.9 Hyperlipidemia	13 (86.6)
t1.10 Diabetes	0
t1.11 Smoking past	12 (80)
t1.12 CAD, 1-/2-/3-vessel disease	3 (20), 2 (13), 10 (66)
t1.13 Previous CABG	7 (46.7)
t1.14 Vein graft, *n*	13
t1.15 Occlude vein graft	9 (69)
t1.16 Previous PTCA	11 (73)
t1.17 Coronary interventions, *n*	25
t1.18 Native coronary obstruction, *n*	18
t1.19 Native coronary occlusion, *n*	22
t1.20 Medication	
t1.21 Aspirin	14 (93)
t1.22 Clopidogrel	4 (27)
t1.23 ACE inhibitor	6 (40)
t1.24 β-Blocker	12 (80)
t1.25 Anticoagulants	2 (13)
t1.26 Channel blockers	6 (40)
t1.27 Statin	8 (53)
t1.28 Nitrates	12 (80)

history, physical examination, and anginal status according to Canadian Cardiovascular Society (CCS); their quality of life was evaluated by the Seattle Angina Questionnaire (SAQ) [6], and the amount of consumed sublingual nitroglycerin per week was taken into account. This evaluation was performed at baseline, days 30, 90, and 180, and 1 year. The cardiac medication was maintained during the follow-up. Dipyridamole ^{99m}Tc MIBI testing protocol quantitative gated SPECT was performed at baseline, days 30 and 90, and 1 year. Coronary angiography was performed at baseline, day 30, and 1 year.

Safety was assessed with laboratory parameters (baseline, 24 h, day 30, and 1 year), ophthalmologic exam (baseline and day 30), time of hospitalization, need of rehospitalization, new symptoms or signs during the follow-up, or new medical condition. During the procedure, tolerance was evaluated clinically and with hemodynamic determination of systemic blood pressure, heart rate, and coronary sinus pressure.

2.1. Bone marrow

Under local anesthesia, BM was aspirated from the iliac crest, mixed with heparin (50 U/ml of BM), and filtered with Bone Marrow Collection Kit (Baxter, IL, USA). Filtered BM was assessed morphologically and tested for the viability, absence of clots, bone spicules, and gross bacterial contamination. The target volume was 60 ml (10 of 15 patients) to 120 ml (5 of 15 patients) with a target cellularity >0.04×10^8/kg. Bone marrow biopsy with CD31 determination by immunohistochemistry studies was performed in all the patients. Serial sections (5 μm in thickness) of BM mounted on 3-aminopropyl triethoxysilane (Sigma)-coated slides were used. Sections were incubated with a monoclonal antibody to CD31 (DAKO Cat.NO. N1596). The visualization of antigens was achieved by the streptoavidin–peroxidase method (Histostain-SP Peroxidase BulkKits-Zymed, USA), and 3,3 diaminobenzidine (Liquid DAB-Plus SubstrateKit-Zymed, USA) were used as chromogen.

This antibody recognized a 100-kDa glycoprotein in endothelial cells and a 130-kDa glycoprotein in platelets. To verify immunoreaction specificity, adjacent control sections were subjected to the same immunohistochemical method replacing primary antibodies by nonimmune serum.

2.2. Bone marrow administration

The BM was infused through the coronary sinus immediately after harvesting. Venous brachial access was used for previous administration of 5000 IU of heparin, and coronary sinus catheterization was performed with continuous electrocardiographic and systemic blood pressure monitoring. For the catheterization and occlusion of the coronary sinus, a balloon catheter of 5/20 to 7/20 or Swan-Ganz 7F was used,

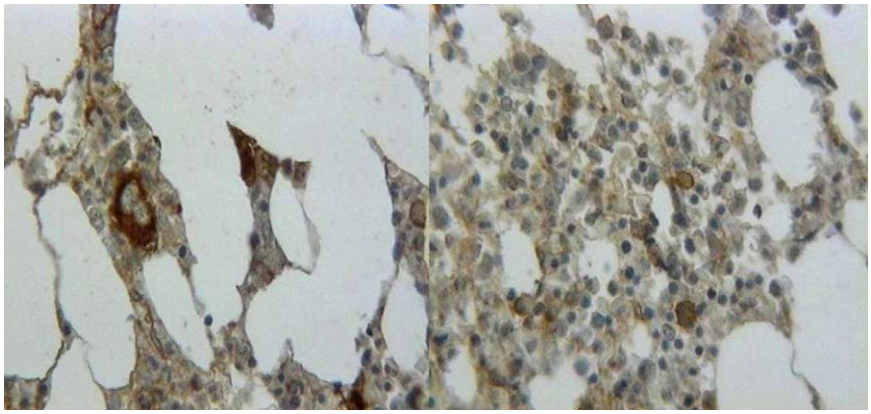

Fig. 2. Immunocytochemistry staining of CD31 in BM biopsy patients, with a quantification of 6.91±1.21% of positive nucleated cells.

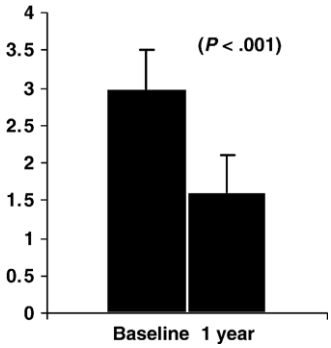

Fig. 3. Canadian Cardiovascular Society angina classification (mean±S.D.).

with determination of the coronary sinus pressure and the pressure during the occlusion (Fig. 1). The BM was administered in 5 min keeping the occlusion of the coronary sinus for 15 min.

2.3. Perfusion protocol

2.3.1. Dipyridamole ^{99m}Tc MIBI testing protocol quantitative gated SPECT

Dipyridamole testing was performed with patients in a fasting state, and all medication was continued. MIBI myocardial tomography was performed with the same-day "stress–rest" imaging protocol. The patient received an intravenous dipyridamole infusion of 0.56 mg/kg in a 4-min period. When angina pectoris, electrocardiographic ST depression, or other medical contraindication was not recorded, another dipyridamole infusion was performed with 0.28 mg/kg in a 2-min period (double protocol). Four minutes after the dipyridamole infusion was completed, 10–15 mCi MIBI was injected. Stress testing was considered abnormal when they had angina pectoris or electrocardiographic ST depression >1 mm. SPECT was performed 45 min later with dedicated single-head imaging system. Six hours after the stress study was completed, 30 mCi MIBI was injected to the patient. Tomographic MIBI imaging was repeated 45 min later with an identical acquisition protocol, but in this opportunity, with acquisition of ECG-gated imaging. An automated left ventricular function analysis software program was used to calculate LVEF.

The left ventricular myocardium was divided into 20 segments. A graded score was applied to the stress and the rest imaging expressing tracer activity, according to the maximal activity found in each study (0=normal, 70–100% of the maximal activity; 1=mild, 50–70%; 2=moderate, 30–50%; 3=severe, 0–30%).

The overall sum of all segments gives the total score of the study. The imaging data were submitted to the core laboratory at Cardiovascular Institute in Buenos Aires, where uniform processing and display was performed. Image interpretation was by consensus of two readers blinded to the type of study (baseline or follow-up) and to the clinical data. The semiquantitative score results from the addition of the score of the different segments in both stress and rest.

2.4. Coronary angiography protocol

Coronary angiography was performed with administration of sublingual isosorbide dinitrate before the study, keeping a constant distance between the table and X-ray tube, with the same degree of oblique, and the collateral vessels were counted using a grid system. The area of interest is identified in the angiogram, and the most representative frame is selected. A grid is then placed over the image and scored according to the number of collateral vessels touching the grid in the frame of interest.

2.5. Statistical analysis

We analyzed the patients' score on the difference scales by using the Friedman's analysis. This test is a non-parametric counterpart of the parametric two-way analysis of variance based on the χ^2 test and was used to test if the

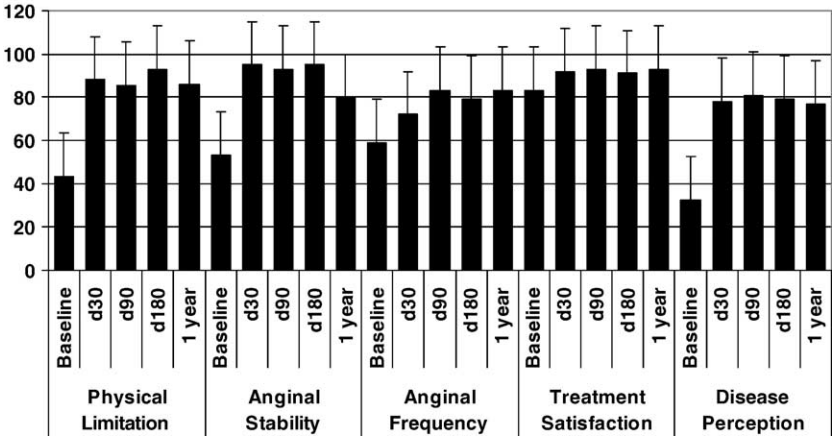

Fig. 4. Seattle Angina Questionnaire, evaluation from baseline to 1 year (mean±S.D.).

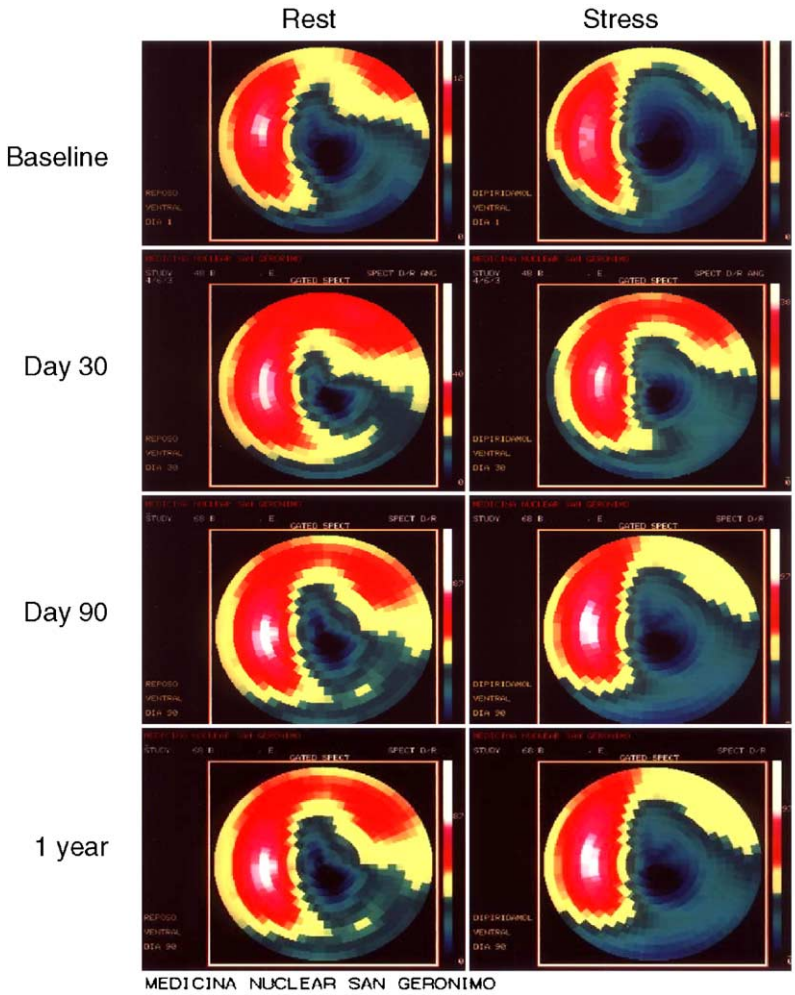

Fig. 5. Baseline showing extensive necrosis anterior, apical, anterolateral, lateral, inferior lateral, and inferior, with ischemia anterior and anterior lateral. At day 30, improvement at rest with a better uptake in the anterior, anterior lateral, and inferior territories; the stress imaging shows improvement at anterior lateral. At day 90 and 1 year, improvement of perfusion in anterior lateral territories continued more at rest than at stress.

medians of the time periods were totally matched when the distribution of the underlying population was not specified. A value of $P<.05$ was considered significant.

3. Results

3.1. Patients' characteristics

Baseline demographic and clinical data of the patients are summarized in Table 1.

3.2. Bone marrow

The filtered BM had no clots or bone spicules and had normal morphology. The infused BM contained 11.48±2.56 cellular count with nucleated fraction of $0.089\pm0.023\times10^{8}$/kg. Bone marrow biopsy disclosed CD31 quantification of 6.91±1.21% of the positive nucleated cells (Fig. 2).

3.3. Procedural and tolerance

All patients were successfully infused with 60 ml (10 patients) and 120 ml (5 patients) of AUBM. No significant arrhythmias or significant changes in blood

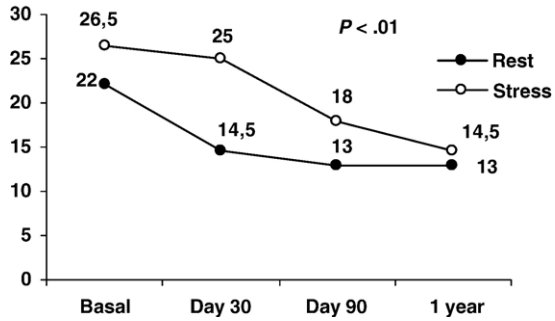

Fig. 6. Myocardial perfusion semiquantitative scoring system at rest and at stress (mean). The average of improvement at rest is 40.9% and at stress 45.3%.

pressure or heart rate was noted during the procedure, with a mean systolic pressure of 134±21, diastolic pressure of 85±10, and heart rate of 64±7. The mean coronary sinus pressure was 13±3 and postinfusion 14±4. The mean coronary sinus pressure during the occlusion was 48±14. The catheterization of the coronary sinus was successful in all the patients. Two patients complained of symptoms, one of them had chest pain that was resolved with sublingual nitroglycerin, and the other one unrelated headache.

3.4. Safety

There were no deaths, myocardial infarction, or worsening of clinical status at 1 year follow-up. Three patients were readmitted into the hospital during the follow-up: one with unstable angina due to self-discontinued antianginal medication, the other one with unstable angina secondary to anemia due to aspirin-induced gastrointestinal bleeding, and the last one due to erysipelas. During the follow-up, one patient was diagnosed with cancer of the lung. After a year of follow-up, the coronary angiography showed progression of coronary stenosis in two patients. All patients were discharged within 24±3 h. The laboratory parameters were within normal range in 14 patients, and mild elevation (1 time upper normal) in the liver enzyme level was observed 24 h after BM infusion in one patient. There were no significant changes in the ophthalmologic exams at day 30.

3.5. Clinical outcomes

The CCS angina classification shows that the mean angina class was 3.0±0.53 at baseline and improved to 2.26±0.59 at day 30, 2.13±0.35 at day 90, 2.0±0.00 at day 180, and 1.6±0.63 at 1 year ($P<.001$) (Fig. 3). Significant improvement at 1 year was also noted in SAQ (Fig. 4): physical limitation, 43.53±21 vs. 86.26±20.6 ($P<.001$); anginal stability, 53.3±35 vs. 80±28.6 ($P<.001$); anginal frequency, 59.3±25.8 vs. 83.3±26.8 ($P<.01$); treatment satisfaction, 83.8±11.2 vs. 93.35±9.5 ($P<.05$); and disease perception, 32.5±15.3 vs. 77.2±20.5 ($P<.001$). An average improvement of 30% in the quality of life at 1 year was noted. Eight patients consumed sublingual nitroglycerin for daily chest pain, and we observed an average reduction of 62% in the number of nitroglycerin tablets consumed per week (47.5 vs. 18). The cardiac medication remained without significant change at 1 year.

3.6. Myocardial perfusion

Semiquantitative analysis with independent core laboratory disclosed that the overall sum of left ventricular perfusion defect score improved at rest and/or at stress in 12 of 15 patients, and 6 of 12 patients had improvement at rest and stress, 3 of 12 only at rest, and 3 of 12 at stress (Fig. 5). The score at "rest" baseline of 9.8±10.6 improved to 8±8.2 at day 30, 7.3±6.9 at day 90, and 7±6.9 at 1 year ($P<.01$); score at "stress" baseline of 19.9±9.9 improved to 14.8±9.8 at day 30, 12.2±8.6 at day 90, and 13.6±7.2 at 1 year ($P<.01$) (Fig. 6). The average of improvement at rest was 40.9% and at stress 45.3%. The score in the stress perfusion defects showed in 9 of 15 patients an improvement of more than 20%, and in 4 of 15 patients, less than 20%; 2 of 15 patients were unchanged, and none of the patients showed worsening in the imaging. At rest, perfusion defects score showed in 9 of 15 patients an improvement of more than 20%, and in 2 of 15 patients, less than 20%; 4 of 15 patients were unchanged, and none of the patients showed worsening in the imaging. The gated ejection fraction baseline of 52±0.13 increased to 57±0.15 at day 30, 58±0.11 at day 90, and 58±0.13 at 1 year ($P<01$).

3.7. Coronary angiography

At 1 year follow-up, the coronary angiography showed more collateral vessels in 10 of 15 patients (Fig. 7). There was no core laboratory evaluation. Progression of coronary

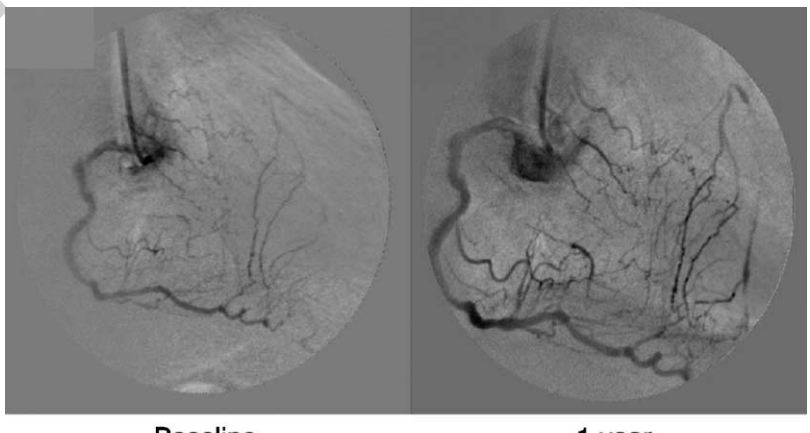

Fig. 7. Coronary angiography of 1 year follow-up. Right coronary artery in RAO projection baseline angiogram and 1 year angiogram with more collateral filling to left anterior descending artery.

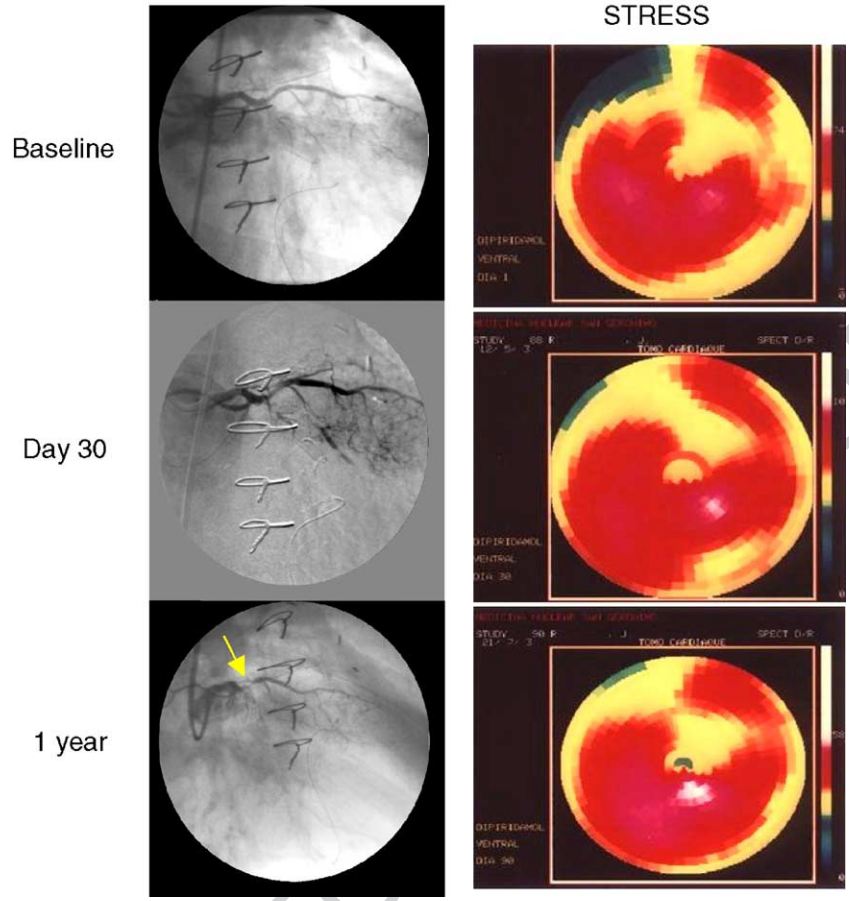

Fig. 8. Progression of coronary stenosis. Baseline image reveals left coronary artery angiogram with occlusion LAD and obtuse marginal branch without significant disease, perfusion representatives of stress polar maps showing severe hypoperfusion of anterior septal, moderate defect of anterior lateral apical, and mild hypoperfusion of lateral basal and inferior lateral. At day 30, angiogram with more collateral filling and improvement of anterior septal, anterior lateral, and lateral basal territories. At 1 year, arrow indicates tight stenosis of obtuse marginal branch, and the perfusion continue like that of day 30, but even with better uptake of inferior lateral.

stenosis at 1 year follow-up of angiography was observed in two patients; one was asymptomatic without deterioration of the perfusion imaging (Fig. 8) and the other one was symptomatic with deterioration of the perfusion imaging due to progressive left main disease from baseline 25% stenosis to 75% stenosis, and required PTCA/stent implantation resulting in clinical and perfusion improvement.

3.8. Readministration of BM

Three patients showed no difference in the perfusion dipyridamole studies (baseline and 1 year). One of them showed significant improvement in the clinical score (SAQ: 62% improvement, CCS Class 3 baseline vs. Class 1 at 1 year); in the other two, the clinical improvement was less significant, and these patients were readministered with 120 ml of BM.

3.9. Comparison between different BM concentration.

Ten patients received 60 ml, and five patients received 120 ml. There were no demographic differences between both groups. The patient who had developed cancer of the lung was in the 120-ml group, and the two patients who had shown progression of coronary stenosis at 1 year follow-up angiography were in the 60-ml group.

4. Discussion

The Phase I clinical study was designed for patients with chronic refractory angina. The number of patients suffering from angina pectoris chronically resistant to conventional treatments is increasing; the prevalence is estimated to be 100,000 patients in the United States, with an equal number in Europe [7]. Patients resistant to conventional treatment may be considered as survivors of their coronary artery disease (CAD) because they are invalided by their anginal pain; without conventional treatment options, these patients have unmet medical needs. Subsequently, any additional treatment that relieves their complaints without adversely affecting their chronic disease is worth taking into consideration.

We have chosen a pragmatic approach by using unfractionated BM cells (BMCs), which contain different stem and

progenitor cell populations, including hematopoietic stem cells, endothelial progenitor cells (EPCs), and mesenchymal stem cells. The mechanisms of how BMCs administration enhance myocardial perfusion are unknown. Bone marrow-derived EPCs have been proposed to enhance tissue perfusion by differentiating into endothelial cells at site of neovascularization [8–10]. Recent articles have highlighted the potential of BMCs to deliver a natural cocktail of angiogenesis and arteriogenic cytokines to the myocardium [11–13].

The experimental background has shown that transendocardial injection of unselected BMCs or EPCs enhances collateral flows, capillary density, and regional contractility in pigs with chronic myocardial ischemia [14]. Our preclinical study of transcoronary sinus delivery of unfractionated BMCs in animal model with myocardial injury induced angiogenesis, as well as vessels with smooth muscles [5]. Also, we have demonstrated that the transitory occlusion of the coronary sinus is well tolerated and, as a route of administration, is effective because BMCs were observed in the myocardium 2 weeks after administration.

The procedure was technically feasible without significant changes in the tolerance parameters. During hospitalization, no significant arrhythmias or worsening of clinical status occurred.

At 1 year follow-up, there was no mortality or myocardial infarction. The three rehospitalizations were due to causes unrelated to the main goal of the treatment. There was a major concern because one patient who had a history of tobacco consumption developed cancer of the lung. We suggest that this may be unrelated to BMC therapy because this was not found in the BM transplantation programs; the incidence of late second malignancies in patients with BM transplantation is more related to the chemotherapy treatment than to the BM transplant [15,16]. Besides, work done by Peters et al. [17] about the "contribution of BM-derived endothelial cells to human tumor vasculature" has shown that such stem cell contributed to tumor endothelium but at low levels, averaging only 4.9% of the total. Another point of view may be the retrograde leak of BMCs from the coronary sinus infusion and homing of BMCs in the lung.

Clinical improvement in the current study is in accordance with the previous trials using growth factors [18]. The improvement of 38% in the quality of life observed at 6 months was maintained at 1 year follow-up (30%). A mean of 1.4 improvement in CCS angina classification was observed at 1 year with a significant reduction in the number of nitroglycerin tablets consumed per week. The improvement in health-related quality of life is an important therapeutic objective for patients with chronic refractory angina. We are aware of the impediment of using quality of life as an objective outcome in clinical research due to the placebo effect. Because this study was designed to test feasibility and safety, we did not include a control group.

Myocardial perfusion imaging and coronary angiography in this study explored mechanisms of symptomatic benefit and provided evidence that such therapy does indeed enhance blood flow to ischemic myocardium. Regarding myocardial perfusion, we observed at 1 year an average improvement at rest of 40.9% and at stress of 45.3% with blinded core laboratory. Improvement at rest in perfusion dipyridamole study is indicative of angiogenesis, and we observed that nine patients improved the resting imaging at 1 year follow-up. The improvement observed in stress imaging might be indicative of more collateral vessels. It is important from a safety point of view that none of the patients showed worsening of imaging. However, the improvement measured may be fortuitous and due to the relatively small number of patients or to the natural biological variability in myocardial blood flow, rather than to the treatment effects.

Collateral vessel improvement by coronary angiography was observed in 10 patients. We considered that the angiography appearance of collateral vessels is limited by the variability of hand injection, spatial resolution of the technique, and, typically, the extensive preexisting collateral networks [19]. Angiography performed at 1 year disclosed progression of the coronary stenosis in two patients; one of them needed treatment with PTCA/stent. One of the concerns of BMC therapy has been the possibility to induce progression of coronary obstruction ("Janus Phenomenon"). On the other hand, it would not be unusual to observe progression of the coronary obstruction in patients with significant CAD.

In conclusion, transcoronary sinus administration of freshly aspirated unfractionated BM in patients with chronic refractory angina with 1 year follow-up is feasible and appears to be safe. Although the results do not prove efficacy, we believe, however, that they do warrant a large randomized controlled study for evaluation of therapeutic efficacy and long-term safety.

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