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Research article

Retrograde Cellular Cardiomyoplasty through the Coronary Sinus. An Experimental Study on Swines

EdvinPrifti[2], Marco Bugetti[1], GuyHarmelin[1], AltinVeshti[1], Andrea Gambacciani[1] and Massimo Bonacchi[1*]

[1]Cardiac Surgery, Department of Experimental and Clinical Medicine, University of Florence – Italy

[2]Division of Cardiac Surgery, University Hospital Center of Tirana - Albania

Abstract

Objectives

The aim was to create a model of myocardial infarction (MI) with a borderline myocardial impairment which would enable to evaluate the retrograde cellular cardiomyoplasty (CCM) through the coronary sinus (CS) in a large animal model.

Materials and Methods

Experience was carried out in 25 juvenile farm pigs, of both sexes, weighing 35 to 40 Kg. 15 were subjected to the procedure, and 10 were used as control. All animals underwent a right antero-lateral thoracotomy on the fifth intercostal space, and the LAD was ligated after the emission of the second diagonal. Almost one month later the animals in the study group underwent a midline sternotomy and a murine myoblastic line C2-C12 in 20cc of medium was injected at a constant pressure of 30 mmHg, with the balloon inflated into the coronary sinus through a Swann-Ganz catheter with a 20 minutes interval. 30 days later all survived animals from the study group and the control group underwent transthoracic echocardiography and 99-Tc MIBI Gated Scintigraphy evaluation and later were euthanized through a midline sternotomy and specimens were taken from the left ventricle for mycroscopic evaluation.

Results

In all animals CPK-Mb, Troponine I and Myoglobin it was seen a progressive rise of CPK-Mb and of Troponine I during the observation period, reaching the maximum values within 12 hours after MI, while Myoglobin reached its maximum level within 4 hours after AMI. Cardiac output decreased significantly after AMI ($p<0.001$), remained low until CCM and increased significantly at the time when the animal was sacrificed versus the values after AMI ($p<0.001$), but comparable to the baseline values (p=ns). Pulmonary artery pressure increased significantly after AMI ($p<0.001$) and improved before the animals' sacrifice versus the post AMI values ($p<0.001$). Instead in the control group such values remained the same at the time of sacrifice compared to the post AMI values. In all animals, the surgical induction of AMI caused a marked decline in the echocardiographic values of cardiac function regarding the LVEF, LVEDV, LVESV and MPI. However the cardiac function and dimensions were significantly improved in the study group versus the control group after CCM. All animals, undergoing CCM, demonstrated a significant reduction of the perfusion deficit in the LAD territory after CCM, instead such data remained unchanged in the control group. The histological examination demonstrated that the engrafted myoblasts could be distinguished from the activated fibroblasts in the scar tissue because they never showed any signs of collagen secretion and fiber buildup. The engrafted myoblasts were mainly found in the AMI area borderline.

Conclusion

Our study provides data in support of the following issues: the venous retrograde delivery route through the CS is safe and effective; CCM provides a significant improvement in function and viability.

Introduction

Cellular cardiomyoplasty (CCM), as a possible substitute treatment of damaged myocardium, has becoming a clinical reality and several reports are today available about the results of direct implant of myoblasts on the scar tissue of the heart [1-4]. In spite of these clinical trials, many questions are still unsolved such as the best cellular substitute, the correct time to engraft the new cells, the best way to administer cells themselves.

At the moment, as previously signaled, clinical studies are evaluating local cells administration as a possible way, but there are experimental studies turned to the evaluation of direct coronary injection [5] and to retrograde venous injection [6-8]. Several other models have been reported in the literature: microspheres injection, intracoronary coils deployment [9,10] leading to an elevated degree of myocardial impairment with a subsequent high mortality rate. This experimental work deals with the possibilities offered by a

***Corresponding author:** Massimo Bonacchi, Cardiac Surgery, Department of Experimental and Clinical Medicine, University of Florence Careggi Teaching Hospital Largo Brambilla, 3 Firenze, Italy, E-mail: mbonacchi@unifi.it

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retrograde venous administration through the coronary sinus(CS) using pigs with moderate left ventricular dysfunction realized by ligature of the left anterior descending coronary artery (LAD) on its middle third.

Aim of this experimental study is to create a model of simple myocardial infarction (AMI) with a borderline myocardial impairment which would allow us to evaluate the retrograde CCM through the CS in a large animal model with low mortality and complications rate and a high reproducibility.

Materials and Methods

Experiment was carried out on 25 juvenile farm pigs, of both sexes, weighing 35 to 40 Kg. 15 were subjected to the procedure, and 10 were used as control. Animals were handled in accordance with the position of the American Heart Association and the European Community Rules on Animal Use for research.

Study Protocol

Phase 1 Induction of AMI in all Animals from Both Groups

Few days before surgery animals on light sedation with intramuscular i.m. Ketamine (0,2 mg/kg) were subjected to Transthoracic Echocardiography and myocardial 99Tc MIBI scintigraphy for basal evaluation of myocardial function and perfusion.

The day of surgery, animals, fasted from the evening before, were sedated with Azaperone (2.5-3 mg/kg), Ketamine (4mg/kg) and Diazepam (0.5 mg/kg) given i.m.. Intravenous access was established through an ear vein and then animals after administration of 1 mg/Kg of Ketamine, were orotracheally intubated and ventilated with a mixture of O_2 and Isoflurane 1-1.5% and N_2O 40%. Peripheral electrocardiogram EKG leads were placed, femoral artery was percutaneously cannulated and signals were recorded on a Marquette Multi channel recorder. Through a small cut-down the right jugular vein was exposed and a Swan-Ganz catheter was advanced into the pulmonary artery for pressures and Cardiac Output (CO) measurements .

Under sterile condition a right antero-lateral thoracotomy on the fifth intercostal space was made, the pleura was entered, lungs were gently retracted and pericardiumwasopened longitudinally and suspended in a cradle. With this approach, the apex of the heart was exposed easily in its infero-lateral aspect. LAD exposure requireed a gentle displacement of the heart towards the surgeon, achieved by a sponge introduced into the pericardial sac. LAD was identified in its middle portion freed from the satelite vein and ligated after the emission of the second diagonal branch on Teflon felt with a 6-0 non absorbable suture (Figure 1A). The extension of the AMI was evaluated with blue metilene infusion in the ascending aorta with a short period of aortic clamping (Figure 1B). Pericardium was then loosely approximated and the chest closed in layers on a chest tube for air evacuation in a standard fashion.

A second evaluation of CO was made after chest closure, then the Swann-Ganz was pulled out and the neck incision was closed.

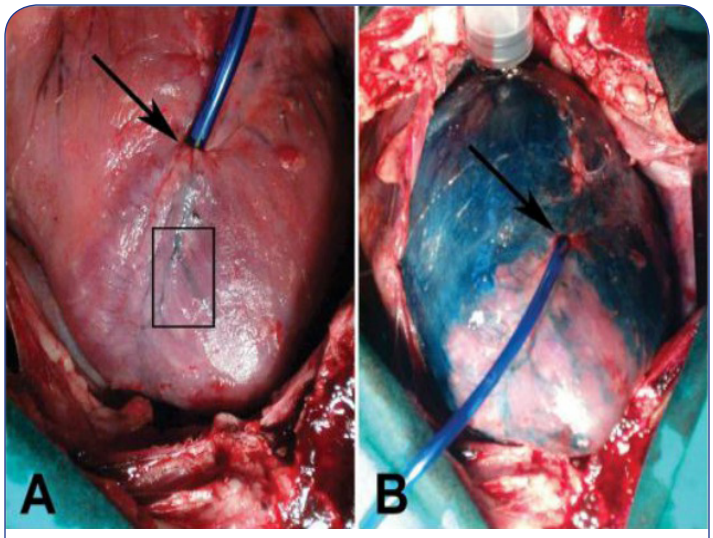


Figure 1: A. Left anterior descending artery ligature through a right antero-lateral thoracotomy. B. Blumetilene injection through the ascending aorta to determine the myocardial infarction area

During the procedure animals received Mg infusion starting from the opening of the chest and continued during all the procedure as well Lidocaine (2 mg/min/Kg). Lidocaine infusion rate was increased in case of severe ventricular arrhythmias and or ventricular fibrillation.

At the end of surgical procedure Furosemide 25 mg, antibiotics and pain medications weregiven, the chest tube was removed, and animals allowed breathing spontaneously. Antibiotics and pain medications were continued for the next 2 days.

Blood samples for myocardial specific enzymes (CK-Mb, Troponine and Myoglobin) were taken and measured in established time intervals after AMI (Table 1).

Phase 2 Follow-Up after AMI

After one month all survivors underwent light sedation for a transthoracic Echo and a second myocardial perfusion evaluation by a 99 mTc MIBI Gated Scintigraphy.

Phase 3 CCM (Only the Study Group)

Few days later the control exams, the animal, under general anesthesia and oro-tracheal intubation, were monitored with EKG and invasive arterial blood pressure detected percutaneously from the femoral artery. Under sterile conditions, a midline sternotomy was performed; the pericardium was opened freeing the adhesions which are usually loose on the superior aspect of the heart. Whilst very tight adhesions were found on the anterior surface of the heart corresponding to the site of LAD ligature. The Pericardium was suspended and a purse string was done on the superior vena cava: a Swann-Ganz catheter was advanced on the pulmonary artery and pressures and CO are detected. The same catheter was then withdrawn from the pulmonary artery and advanced through the coronary sinus to the anterior descending vein, parallel to the

Table 1: Biochemical laboratory data

Group I	Baseline	4 hours	8 hours	12 hours	1 week	24 hours post cardiomyoplasty
Troponin I (µg/l)	0.06 ±0.052	15.1 ± 10.2	36.6±15.6	44.8±17.4	0.36 ± 0.01	0.442 ± 0.085
CK-MB (ng/ml)	0.03 ±0.01	0.072 ± 0.012	0.19±0.058	0.26 ± 0.09	0.08 ± 0.015	0.068 ± 0.013
Myoglobine (ng/ml)	33.5±20	696±345	580±314	422±213	40±21	36±15

Control Group	Baseline	4 hours	8 hours	12 hours	1 week	At time of CCM in Group I
Troponin I (µg/l)	0.04 ±0.01	13.4 ± 7.5	39±17	47±21	0.3 ± 0.012	0.12 ± 0.05
CK-MB (ng/ml)	0.026 ±0.01	0.066 ± 0.01	0.24±0.04	0.28 ± 0.02	0.073 ± 0.02	0.055 ± 0.017
Myoglobine (ng/ml)	31±12	678±331	601±355	445±254	38±19	33±18

homonymous artery (Figure 2A).

Pressure were registered with the inflated balloon and then a murine myoblastic line C2-C12 in 20cc of medium was injected at a constant pressure of 30 mmHg, with the balloon inflated through a 20 minutes interval. The catheter was then flushed with saline and withdrawn. During the injection no arrhythmic problems were observed. Pericardial remnants were then loosely closed; sternum, muscular and subcutaneous layers were approximated with non absorbable sutures in separate layers.

Animal's werenursed with pain medications, antibiotics for 3 days and cyclosporine was given orally (3 mg/Kg) until the animals were sacrificed.

In most studies evaluating myoblast transplantation, immunosuppressive therapy has been used to improve cell acceptance and survival. In particular, cyclosporine prevents donor-cell rejection [11,12]. Similar results have been reported for allo and xenomyoblast transplantation [13]. Therefore, because of the non-isogenic cell origins and immunogenic *eGFP* expression, all the animals undergoing cardiomioblasty received immunosuppressive therapy: a daily dose of 15 mg/kg of cyclosporine with food, beginning on day 1 of the administration of cells or placebo [14].

Phase 4 Follow-Up after CCM

30 days later all survived animals from the study group and the control group underwent transthoracic echocardiography and 99 Tc MIBI Gated Scintigraphy evaluation and later were euthanized through a midline sternotomy (Figure 2B) and specimens were taken from the left ventricle for mycroscopic evaluation.

Generation And Culture of Engineered C2C12 Myoblast Cell Lines

Mouse skeletal C2C12 myoblasts (ATCC, Manassas, VA, USA) were cultured in DMEM containing 10% fetal bovine serum (Sigma, Milan, Italy). Cells were transduced with an integrating lentiviral vector bicistronically expressing human preprorelaxin 2 cDNA [15] and eGFP gene, or just eGFP, under a cytomegalovirus (CMV)

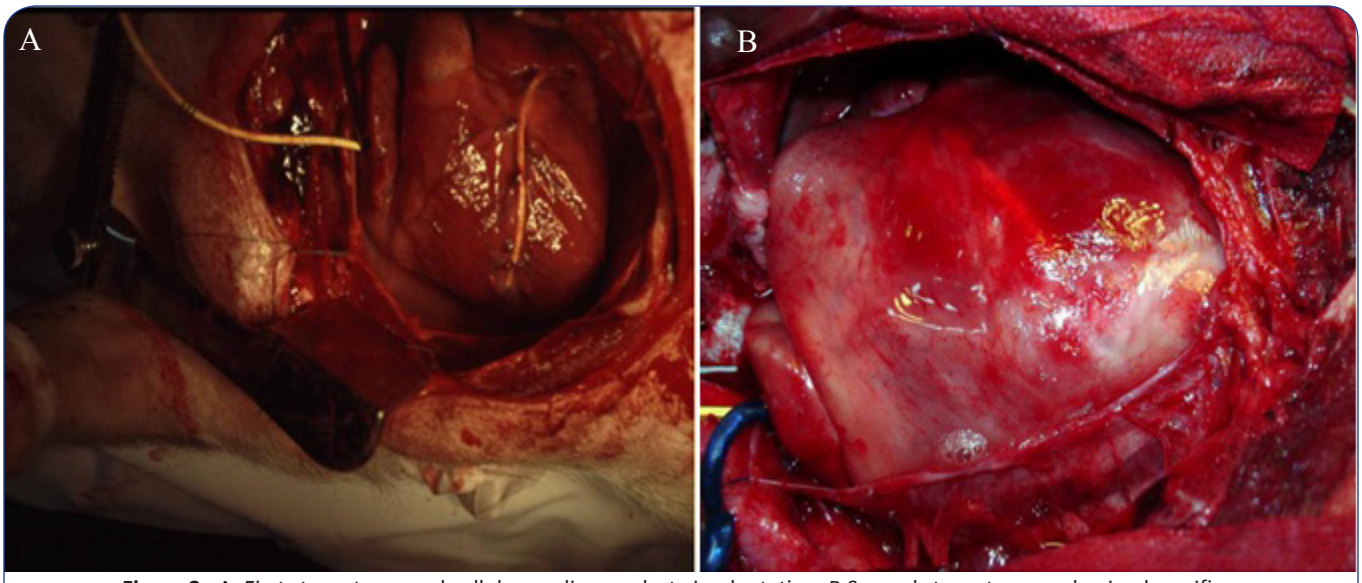


Figure 2: A. First sternotomy and cellular cardiomyoplasty implantation. B Second sternotomy and animal sacrifice

promoter. These cell strains were termed C2C12/RLX and C2C12/GFP, respectively. Clones were selected by cloning ring method and analyzed by fluorescent microscopy and flow cytometry for eGFP expression. Cells were grown in DMEM, containing 10% fetal bovine serum (Sigma) and 0.1% gentamycin, in a 5% CO2 atmosphere at 37°C. When required for transplantation, cells were detached using EDTA 0.1% in phosphate-buffered saline (PBS) and mechanical scraping, centrifuged and washed twice in PBS and finally suspended in complete culture medium, as described below. Cell concentration was determined using a Burker chamber and adjusted to the amount required for individual injections.

Echocardiography

A Transthoracic ECHO using a Philips Envisor ECHO with a 3.5 MHz phased array transducer was used. Evaluations were done on M-mode, B-mode, continuous wave and Doppler recordings.

M-mode measurements were made according to the guidelines of the American Society of Echocardiography, from right parasternal long axis view; mitral and aortic flows were recorded from the apical four and five chamber views. The following parameters were evaluated:

left atrial diameter, left ventricular end diastolic and systolic diameters (LVEDD and LVESD respectively), fractional shortening (FS), Left ventricular Ejection fraction (LVEF) according to Simpson method. Myocardial performance index (MPI), as the sum of isovolumic contraction and relaxation times, was also evaluated. The sum of isovolumic contraction and relaxation times was derived from the interval between the end of mitral inflow and the onset of the next mitral inflow signal minus ejection time, evaluated as the interval between the beginning and the end of aortic flow.

Myocardial Scintigraphy

All animals underwent evaluation of myocardial perfusion using a 99Mc Tc MIBI gated SPECT which enables evaluation of myocardial perfusion on three different projections: short axis, vertical long axis and horizontal long axis.

Data were acquired on Picker Prism 3000 XP and CEqual Cedar Emory quantitative analysis software using a specific cardiac stress SPECT protocol at a frequency of 20 sec. per frame.

99 M Tc. binded with MIBI was injected through an ear vein at a dosage of 0.5miCu. / Kg. Animals were kept in a separate cage for the first 3 days to allow the dismission of the nuclear activity.

From the data obtained a reconstruction was possible with evaluation of the volumes (end-systolic and end-diastolic) and evaluation – determination of Ejection Fraction.

Histologic Analysis

To study the distribution and differentiation of myoblasts, were examined the eGFP and sarcomeric D-actin expression by immune fluorescence (10μ-thick myocardial samples fixed in formaldehyde vapors for 10min and then incubated with rhodamine conjugated monoclonal antibody anti-eGFP and antisarcomeric D-actin). Other sections were examined for immune reactions by means of incubation with polyclonal antibodies anti-VCAM-1 and ICAM-1. The immune reaction was detected with use of a confocal laser scanner microscope that could detect secondary antibodies. Ultrathin sections of myocardial tissue samples, fixed in glutaraldehyde and osmium tetraoxide of epoxy resin, were put on a slide with uranyl acetate and citrate and examined under electron microscopy. Some fragments, not fixed in osmium, were used for immune electron microscopy to reveal the relaxin produced in the grafted C2C12-relaxin cells by anti-H2-relaxin antibodies. Fibrosis in the cardiac tissue was studied by means of morphologic analysis of samples of tissue, fixed in paraformaldehyde and paraffin. Some sections were stained in accordance with the Van Gieson method for evaluation of collagen (0.1% fuchsin in hydrogen peroxide containing 0.08% hydrochloric acid for 4 min, and then washed in 95% ethanol for 5 min) and analyzed by means of optical microscopy.

Statistical Analysis

Group statistics were expressed as mean ± SD. Fisher's exact test was used for the non continuous variables. T student test was employed for the continuous variables. Significance between data was considered achieved when $p<0.05$.

Results

Survival Rate

2 animals from the control group died in the immediate post-operative course due to irreversible Ventricular Fibrillation not responsive to Lidocaine injection and DC shock. Another animal died in the control group after the AMI. All other animals from the control group completed the protocol and were sacrificed with humanitarian's method after 30±2 days from sternotomy EKG changes: immediately after the ligature a progressive elevation of the ST segment was observed which remains stable during the surgical procedure; at 1 month an anterior q wave was present in all animals.

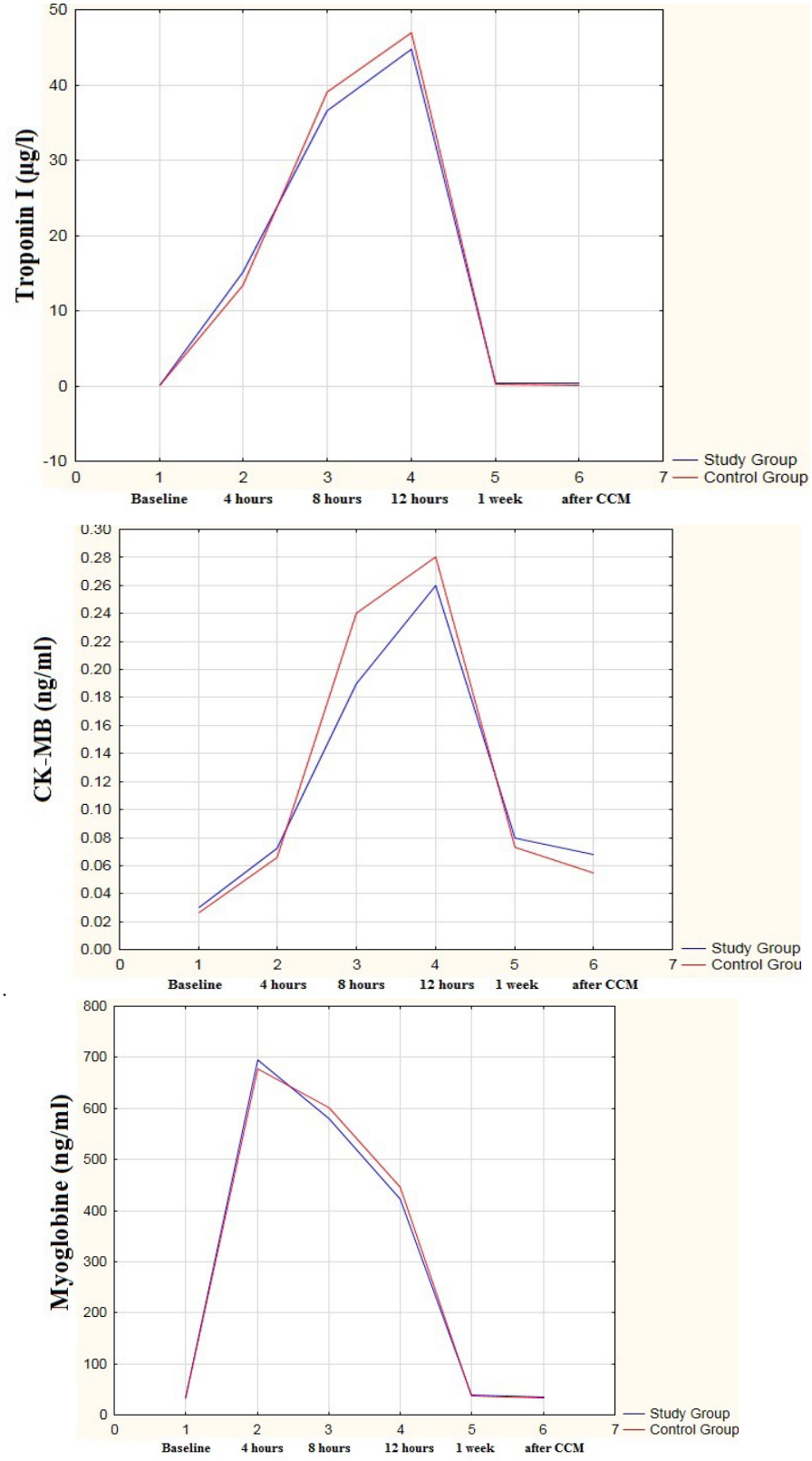
Biochemical Markers

In all animals CPK-Mb, Troponine I and Myoglobin it was seen a progressive rise of CPK-Mb and of Troponine I during the observation period reaching the maximum values within 12 hours after AMI while Myoglobin reached its maximum level within 4 hours after AMI (Figure 7).

Hemodynamic Parameters

Cardiac output decreased significantly after AMI ($p<0.001$), and remained low until CCM and increased significantly at the time when the animal was sacrificed versus the values after AMI ($p<0.001$), but comparable to the baseline values (p=ns). Pulmonary artery pressure increased significantly after AMI ($p<0.001$) and improved before the animals 'sacrifice versus the post AMI values ($p<0.001$) (Table 2). Instead in the control group such values remained the same at the time of sacrifice compared to the post AMI values.

Figure 7: Distribution of Troponine I level (A), CK-MB (B) and myoglobine (C) between the study and control group.



Echocardiographic Findings

Table 3 shows the average echocardiographic values in each group at each time point and quantifies the statistical significance of the differences. In all animals, the surgical induction of AMI caused a marked decline in the echocardiographic values of cardiac function. However the cardiac function and dimensions were significantly improved in the study group versus the control group after CCM.

Myocardial Scintigraphy 99Tc Spect

Of the 13 survivors in Group I only 12 animals completed the Myocardial scintigraphy: one was lost because the animal shivered during the procedure and the acquisition of the images was not satisfactory, therefore they were discharged. All 9 survivors in control group completed the myocardial scintigraphy.

All 13 survivors demonstrated a significant reduction of the perfusion deficit in the LAD territory after cardiomyoplasty, instead such data remained unchanged in the control group. All patients in the study group underwent qualitative evaluation (Figure 3A), quantitative evaluation (Figure 3B) and functional evaluation (Figure 3C) after AMI and the same evaluation before the animal's sacrifice (Figure 4).

Histologic Results

In the animal control group, the Van Gieson analysis demonstrated the presence of a scar area with connective tissue, with absent vascularization. Confocal microscopic immunofluorescence revealed absence of GFP positive cells.

We used antibodies for GFP, sarcomeric D-actin, to identify and locate the implanted cells in the myocardium. Confocal microscopic immunofluorescence analysis revealed numerous GFP positive cells. The engrafted cells were mainly located in proximity of blood vessels, principally around the postcapillarvenules, in the periphery of the infracted myocardium and as isolated elements inside the infarcted area, whereas they were not detected in the viable myocardium and not involved in the postinfarction remodeling process (Figure 5). The presence of such cells was confirmed by their positivity versus the sarcomeric D-actin expression.

In small blood vessels within the infarcted area beside myoblast colonies, wewere found endothelial cell adherence markers, intercellular cell adhesion molecules and vascular cell adhesion molecules which were not expressed in non ischemic myocardial tissue. Analysis of vascular endothelial growth factor expression

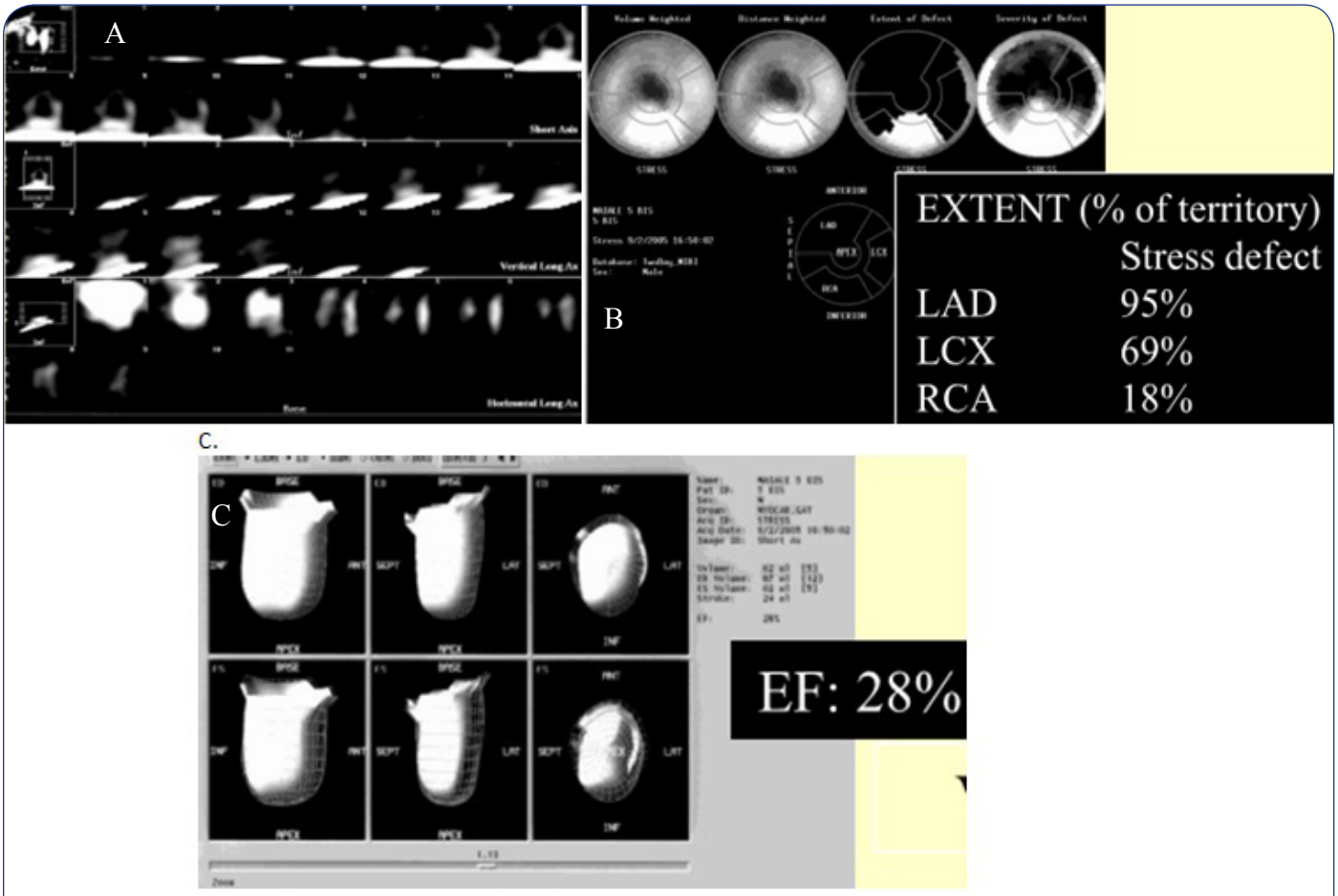


Figure 3: Myocardial scintigraphy evaluation after the myocardial infarction in Animal 1 A. Qualitative evaluation. B. Quantitative evaluation. C. Functional evaluation.

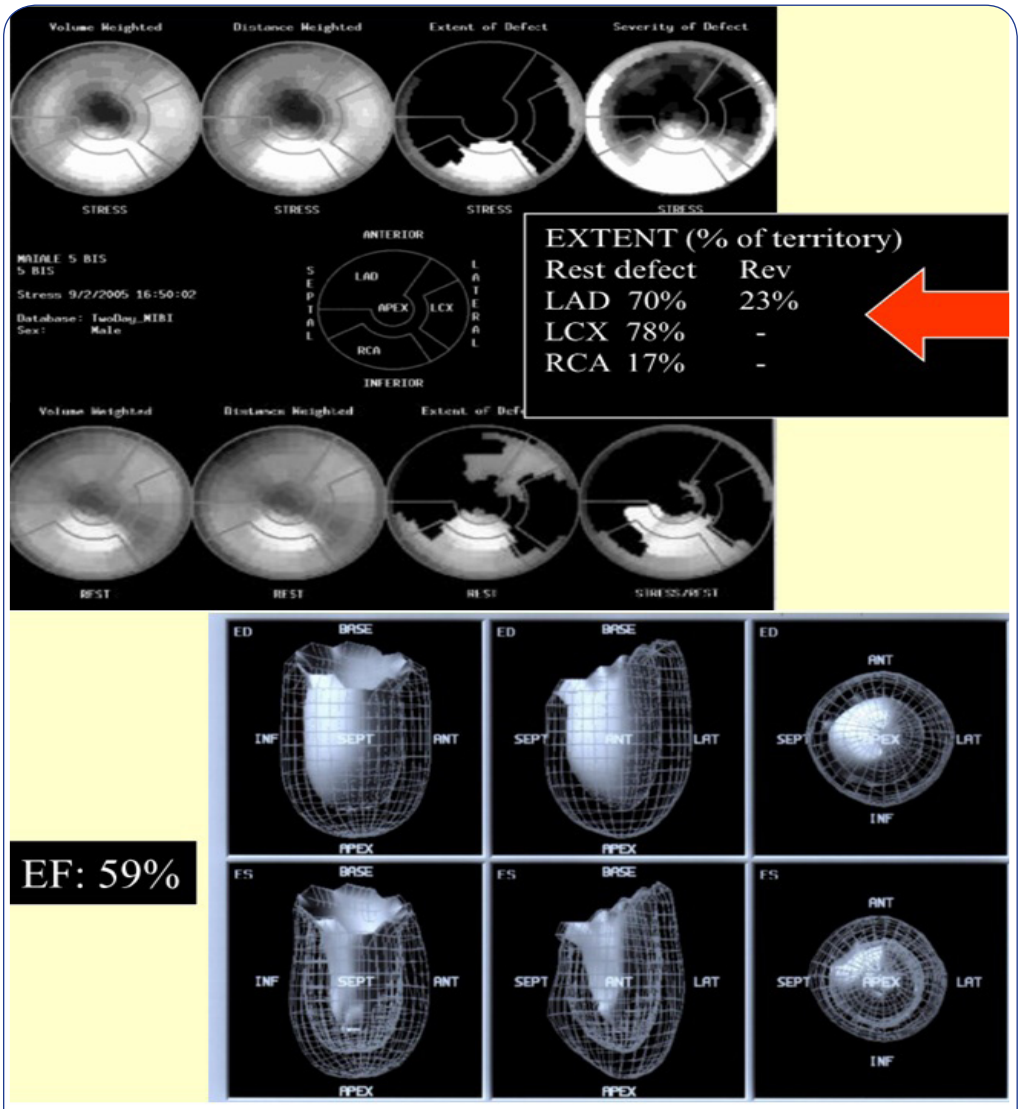


Figure 4: Myocardial scintigraphy evaluation after cellular cardiomyoplasty in Animal 1 A. Quantitative evaluation. B. Functional evaluation.

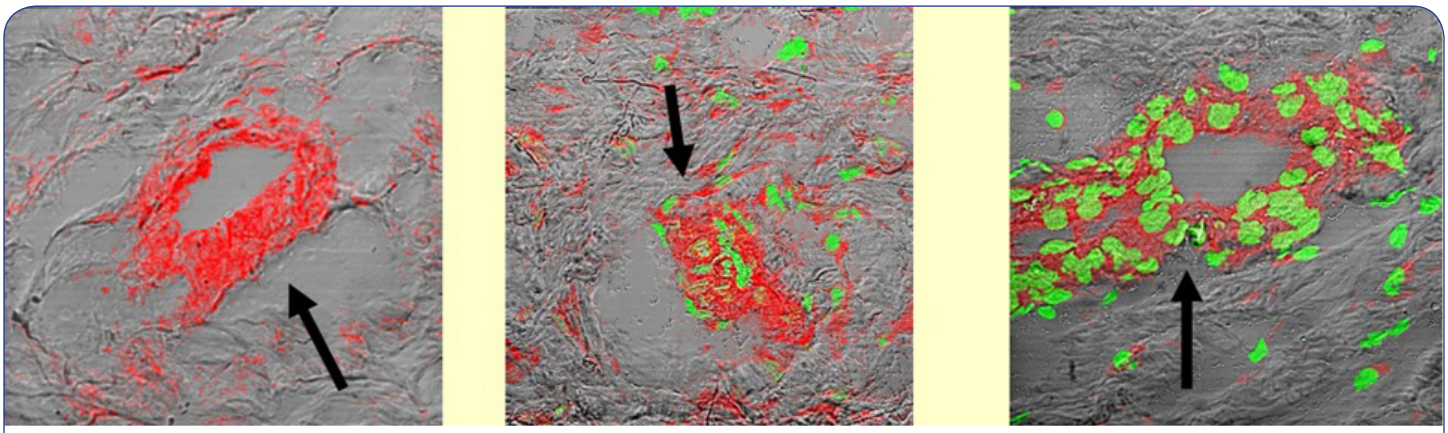


Figure 5: Presence of engrafted myoblasted around the vessels.

Table 3: Echocardiographic and scintigraphic data

	Basal	post AMI p-value	post CCM	p-value	Versus Basal	Versus post AMI
STUDY GROUP						
Echocardiographic data LVEDV (ml)	54.8±8.8	67.1±11.7	0.006	58.7±8.5	0.26	0.047
LVESV (ml)	29.7±4.2	39±9.6	0.004	27.8±12.6	0.61	0.018
LVEF (%)	47.6±5.8	35.2±6.8	0.001	42±8.7	0.07	0.03
FS	31±0.8	25±5.7	0.001	28.8±6.7	0.25	0.132
MPI	0.28±0.09	0.69±0.29	0.001	0.40±0.22	0.08	0.008
Myocardial scintigraphy data						
Perfusion deficit in LAD territory	34±9	37±8	0.44			
LVEF	31±8	28±5	0.33			

	Basal	post AMI p-value	post CCM	p-value	Versus Basal	Versus post AMI
STUDY GROUP						
LVEDV (ml)	56±6	66±10	0.014	68±8	0.01	0.63
LVEDS (ml)	29±5	45±8	0.001	47±11	0.001	0.65
LVEF (%)	50±7	33±5	0.001	30±4.5	0.001	0.2
FS	30±1	24±3	0.001	23.5±3	0.001	0.7
MPI	0.28±0.1	0.66±0.2	0.001	0.61±0.16	0.001	0.57
Myocardial scintigraphy data						
Perfusion deficit in LAD territory	34±9	37±8	0.44			
LVEF	31±8	28±5	0.33			

Legend: LVEF- Left Ventricular Ejection Fraction, LVEDV-Left Ventricular End Diastolic Volume, LVESV-Levt Ventricular End Systolic Volume, FS-Shortening Fraction, MPI- Myocardial Performance Index, AMI-Acute Myocardial Infarction, LAD-Left Anterior Descending Artery, CCM_ CellularCardiomyoplasty

Table 2: Hemodynamic parameters

Study Group	Baseline	After AMI	P-baseline	Before CCM	P-after AMI	P- baseline	Prior animal sacrifice	P-before CCM	P- baseline	P- after AMI
CO (L/min)	6.86 ±0.73	5.3 ±0,83	0.001	5.9 ±1.1	0.13	0.015	6.77 ±0.9	0.037	0.78	0.001
PAP (mmHg)	18.85 ±2.5	26.7 ±5.8	0.001	24.5 ± 4	0.27	0.001	19.5±3.3	0.002	0.58	0.001
Control Group										
CO (L/min)	6.75±1	5.2 ±1.2	0.019				5.6 ±0.7		0.012	1
PAP (mmHg)	17 ±3	26 ±6	0.001				23.8 ±2.5		0.001	0.33

Legend: CO-Cardiac Output, PAP-Pulmonary Artery Pressure, AMI-Acute Myocardial Infarction, CCM-Cellular Cardiomyoplasty

enabled us to evaluate the effects of cardiomyoplasty on scar tissue vascularization. The engrafted myoblasts could be distinguished from the activated fibroblasts in the scar tissue because they never showed any signs of collagen secretion and fiber buildup. The engrafted myoblasts were mainly found in the AMI area borderline (Figure 6).

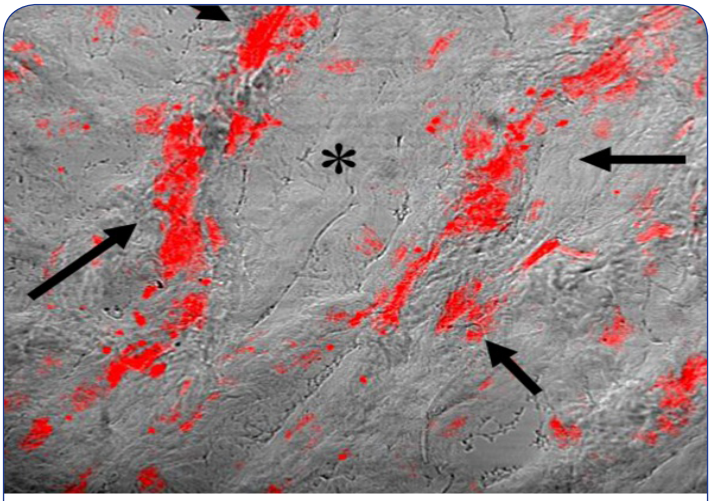


Figure 6: Photomicrograph showing C2C12-GFP and C2C12-relaxin myoblasts grafted in the postinfarction cardiac scar tissue.

Discussion

The loss of cardiomyocytes and contractile units after AMI leads to acute dysfunction and eventually to congestive heart failure. The regenerative potential of myocardium is not adequate to restore the dead tissue [16]. The structural changes (remodeling) are accompanied by functional alteration that leads to deteriorating ventricular function and the worsening of congestive heart failure.

Several types of cells have been proposed for CCM [17-19]. We have been chosen heterologous mouse myoblasts because they are more resistant to ischemia than most other cells and are easy to harvest and expand in cultures [8-20,21]. Myoblasts appear to be capable of settling into host tissue and contributing to function; however, they do not completely differentiate into cardiomyocytes [22,23]. The electrical properties of skeletal myoblasts differ from those of skeletal muscle cells and from those of cardiomyocytes; the resultant functional improvements are probably due to mechanisms other than electromechanical coupling with the host tissue [24]. Skeletal myoblasts are thought to exert their beneficial effect by remodeling the extracellular matrix and secreting cytokines and growth factors that induce angiogenic stimuli [25-27].

CCM is becoming a very appealing procedure for the treatment of failing myocardium, but one of the problems still to be solved is the choice of the way of cell delivery accordingly to achieve the best engrafment possible of cellular elements in the fibrotic tissue.

Up to date two roads have been studied and clinically tested: direct injection into the myocardium associated with CABG and injection into the coronary artery during AMI associated with PTCA [2-28]. Other attempts have been made also by direct injection through the endocardium with evidence of the dysfunctional zone by the Noga system [29], or through a catheter advanced into the CS to the vein effluent from the infarcted zone [31,32].

Results from clinical trials up to now have raised "more questions than answers", leaving room for searching other alternatives. Use of the CS and the coronary veins, as a way of direct administration of stem cells, has been shown to be feasible by Suzuky et al [6,7], however such studies were performed in rats only [8-20]. Similar studies in larger animals are necessary to demonstrate the functionality of such a procedure in humans.

Coronary anatomy and heart physiology in small animals are different from humans; therefore not all results can be translated in the human field. Bigger size animals currently available are dogs, which are not suitable for this kind of experiments because of the rich collateral circulation, sheep and pigs. Sheep has been extensively used to reproduce model of heart failure but coronary circulation is somehow different because the supply of the left ventricle is granted from a LAD, which is named left homonymous artery, giving up to 2 major diagonal branches and, after this, only small branches arise from the artery, making it difficult to perform a ligature to achieve a uniform AMI without an important ventricular dysfunction. The bovine model of post infarction dilated myocardiopathy is a very appealing procedure but to our purposes it has some drawbacks: procedure is somehow cumbersome requiring two general anesthesia and several turns of light sedations with surgical incisions for echocardiographic evaluation. In case of a subsequent operation for CCM, tight adhesions are to be expected on the left ventricle. Pigs' left side coronary anatomy is quite similar to the human as shown before and further more there is lack of important collateral vessels in the common situation.

LAD occlusion, before the emission of the third diagonal branch, creates an infarction, which is about 30-35% of the anterior left ventricular wall as shown by methylene blue dye injection and its extension is quite uniform.From the hemodynamic stand point, it achieves a reduction on heart performance as indicated by a slight reduction on cardiac output and moderate raise on the capillary wedge pressure, without hampering performance fatally and avoiding an overt picture of cardiac failure.

In our model, the right anterior thoracotomy would limit, at the time of the re-operation, adhesions and the assessment of myocardial contractility by Echo, as well of myocardial perfusion by 99Mc TcMibi, thatrequires only a light sedation achievable by intramyocardial injection.

Absence of indexes of overt myocardial dysfunction, such as weight loss and dyspnea, are not seen in our model, but we have to remember that animals live a very steady life and do not exercise too much;but myocardial perfusion images, obtained by scintigraphy, are consistent with an important deficit of perfusion on the anterior wall and apex, leading to a reduction on the calculated LVEF.

The LVEF evaluation demonstrated that, without therapy, functional myocardial recovery would not have been significant. In particular, CCM led to better results in the study group versus the control group, by a significant increase of the LVEF after CCM.

The LVEDD and LVESD results suggest that, without therapy, the ventricular chamber reaches a diameter that does not improve in subsequent determinations. The expansion progressively decreased

in the group undergoing retrograde CCM, reaching final values not much different from those at baseline.

The MPI was inversely proportional to left ventricular functionality and almost tripled after AMI. It then remained constant over time in the control group, so there was not a substantial recovery in myocardial function in the absence of therapy. CCM resulted in a clear and steady improvement.

The echocardiographic results showed that the CCM resulted in left ventricular geometric global and segmental kinetic improvement, probably with a positive influence on remodeling.

The myocardial scintigraphy analysis showed that CCM decreased the perfusion deficit in the LAD territory and led to a significantly better left ventricular function measured simultaneously. This result can be explained by assuming that myoblasts administered via retrograde venous delivery overcome the endothelial barrier, settle in the extracellular matrix, and survive. The evolution of myocardial viability suggests a myoblast replication [6,7-32] or, alternatively, myoblast homing could encourage resident cells to proliferate through a paracrine action [1-33,34]. The myoblasts can settle into scar tissue and proliferate faster and better.

Histologic evaluation showed that the administered myoblasts were located selectively in affected myocardial tissue, as documented by immunofluorescence distribution of their markers. As others did [6,7], we noticed that myoblasts delivered via the retrograde venous route colonized the infarcted tissue effectively. Histologic studies clarify that myoblasts tend to settle mainly in post-capillary venulaeat the edge of the scar, where the endothelium expresses markers of cell adherence, which are absent in the healthy myocardium. This suggests a role of activated endothelium in promoting the adhesion and diapedesis of administered cells, encouraging their location in the injured tissue.

Conclusion

Our study provides data in support of the following issues: the venous retrograde delivery route through the CS is safe and effective; CCM provides a significant improvement in function and viability of the infarcted myocardium.

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