Laboratory Investigation

A Dosing Study of Bone Marrow Mononuclear Cells

for Transendocardial Injection in a Pig Model of Chronic Ischemic Heart Disease

Guilherme V. Silva, MD
Marlos R. Fernandes, MD
Cristiano O. Cardoso, MD
Ricardo R. Sanz, MD
Edie M. Oliveira, MD
Pilar Jimenez-Quevedo, MD
Javier Lopez, MD
Franca S. Angeli, MD
Yi Zheng, MD
James T. Willerson, MD
Emerson C. Perin, MD, PhD

We studied the effect of the dose of bone marrow mononuclear cells, delivered via transendocardial injection, upon capillary density and fibrosis in pigs with chronic ischemic heart disease.

Pigs (n=16) that had undergone ameroid constrictor placement (left circumflex coronary artery) to induce chronic ischemia were divided equally into 4 groups on the basis of bone marrow mononuclear cell dose: control (saline injection) and 50, 100, or 200 × 10^6 bone marrow mononuclear cells. Thirty days after ameroid placement, each pig received 13 transendocardial NOGA-guided injections. An implantable loop recorder monitored possible arrhythmias caused by cell transplantation. Thirty days later, the pigs were killed, and their hearts were evaluated histopathologically for fibrosis and capillary density; the number of cells per segment was correlated with fibrosis and capillary density. No adverse events, arrhythmias, or cardiac inflammatory reactions were associated with cell therapy. Less fibrosis was seen in pigs that received 100 × 10^6 cells than in control pigs. A trend toward higher capillary density was seen with higher cell concentrations. Segments injected with more than 20 × 10^6 million cells had the highest capillary density and the least amount of fibrosis (P <0.05 vs controls).

In conclusion, transendocardial injections (up to 200 × 10^6 bone marrow mononuclear cells) were safe. Analyses of individual injected segments suggest potential benefit from higher cell concentrations per segment. ***(Tex Heart Inst J 2011;38(3):219-24)***

Key words: *Animals; bone marrow transplantation; myocardial ischemia, chronic/therapy; pigs; prospective studies*

From: *Stem Cell Center, Texas Heart Institute at St. Luke's Episcopal Hospital, Houston, Texas 77030*

Dr. Cardoso is currently with the Instituto de Cardiologia do Rio Grande do Sul (RS-Brazil); Dr. Sanz, with the Department of Cardiology, Hospital General Universitario Gregorio Marañón (Madrid, Spain); Dr. Oliveira, Instituto do Coração da Serra Gaúcha Ltda. (Caxias do Sul, Brazil); Dr. Jimenez-Quevedo, Hospital Clínico San Carlos (Madrid, Spain); Dr. Lopez, University Hospital (Valladolid, Spain); and Dr. Angeli, the University of California, San Francisco (San Francisco, California).

Address for reprints: *Guilherme V. Silva, MD, 6624 Fannin St., Suite 2220, Houston, TX 77030*

E-mail: *guilhermesilva@sbcglobal.net*

Studies have shown that bone marrow mononuclear cells (BMMNCs) have the potential to improve the perfusion of chronic ischemic myocardium.[1-6] Transendocardial injection using electromechanical mapping (EMM) has been performed safely in preclinical and clinical studies.[7-9] Because it can distinguish viable ischemic tissue from normal and infarcted myocardium, EMM can be used to target sites in the heart for cell injection.[10-12] In addition, EMM-guided transendocardial injections can be clustered, which enables higher localized tissue concentrations of stem cells.[13]

Despite mounting preclinical and clinical evidence of the beneficial effects of cell-based therapy, optimal cell dosing and delivery approaches have not been identified. A clinical study of patients with acute myocardial infarction (AMI), in which BMMNCs were delivered via intracoronary infusion, suggested a dose-dependent effect in regional myocardial improvement.[14] An experimental study in a chronic heart failure model in rats, however, raised the concern that intramyocardial injections of bone marrow cells could induce ventricular arrhythmias during the first 2 weeks after cell delivery.[15]

The present study was designed to examine the effect of the transendocardial injection of 3 different doses of BMMNCs in a pig model of chronic myocardial ischemia. The occurrence of arrhythmias was monitored in all groups by implantable loop recorders. In addition, we sought to determine, on the basis of histopathologic analy-

At the time of this study, Dr. Perin was on the Scientific Advisory Board of the Cordis Corporation and was a consultant for Biologics Delivery Systems, but he no longer holds those positions. He currently serves on the Stem Cell Advisory Board for Johnson & Johnson. This study was funded by Cordis Corporation, a Johnson & Johnson company.

ses, the optimal approach to transendocardial injections and the establishment of a dosing threshold.

Materials and Methods

This study was reviewed and approved by the Texas Heart Institute Institutional Animal Care & Use Committee and met the criteria of the National Institutes of Health and the American Heart Association (AHA) guidelines for animal research. The study was conducted in our cardiovascular research laboratory.

Study Design

For this study, 16 male and female pigs, weighing between 40 and 60 kg, underwent coronary angiography, followed by placement of an ameroid constrictor to induce ischemia during open-chest surgery (day 0). At 30 days after surgery, all pigs again underwent coronary angiography, followed by the harvesting of bone marrow, from which BMMNCs were isolated. The pigs were randomly assigned into 4 groups: a control group and 3 different dose groups. The BMMNCs were delivered via EMM-guided transendocardial injections into the hearts of the 3 dose groups: 50×10^6 BMMNCs (n=4), 100×10^6 BMMNCs (n=4), or 200×10^6 BMMNCs (n=4). The control group (n=4) received transendocardial injections of saline solution. At 60 days after surgery, all pigs were humanely killed, and histopathologic analyses of the hearts were performed.

Arrhythmia Surveillance

Serial electrocardiograms were recorded at baseline, during the injection procedure, and at follow-up (day 60). An implantable loop recorder was inserted subcutaneously under the left scapula immediately after ameroid placement to check for malignant ventricular cardiac arrhythmias. Heart rhythm was noninvasively monitored via the recorder weekly, every 4 to 7 days, until the pigs were killed at day 60.

Chronic Ischemia Model

A left thoracotomy was performed after inducing anesthesia with the use of tiletamine/zolazepam (4 mg/kg, intramuscularly) and atropine sulfate (0.04 mg/kg, intramuscularly) and was maintained with isoflurane (0.5%–3%) or isoflurane and GKX (guaifenesin 50 mg/mL, ketamine 1 mg/mL, and xylazine 0.01 mg/mL), and oxygen (40%–100%). Chronic myocardial ischemia was produced by placing an ameroid constrictor (Research Instruments SW; Escondido, Calif) around the proximal left circumflex coronary artery (LCx).

Coronary Angiography

At days 0 and 30, left selective coronary angiography was performed by using a 5F Amplatz 1 catheter under fluoroscopic guidance to document baseline patency, adequate ameroid placement, and impaired coronary flow to the LCx. The pigs underwent the BMMNC injection procedure only if impaired coronary flow to the LCx was documented. All 16 pigs received injections.

Cell Harvesting and Isolation Procedures

At 30 days after ameroid placement, the pigs were anesthetized as previously described and placed in ventral recumbence. From the iliac crest, 100 mL of bone marrow was harvested. The BMMNC fraction was isolated by using Ficoll density gradient centrifugation with Ficoll-Paque™ PLUS media (GE Healthcare; Piscataway, NJ) as previously described.[3] A solution of saline with heparin and 5% albumin was used to wash and remove the cell aggregates. The cells were resuspended in 5% albumin-saline solution, counted, and tested for viability. The final samples were stored in 10-cc syringes and transferred to 1-cc syringes at the time of injection. The pigs were treated only with viable cell products (>95% viability).

Electromechanical Mapping Procedure

After BMMNC harvesting on day 30, EMM was performed using the NOGA® 4.0 system (Biologics Delivery Systems Group of Cordis Corporation, a Johnson & Johnson company; Irwindale, Calif), as previously described.[16,17] In brief, the pigs were administered heparin (70 U/kg) and underwent biplane left ventricular (LV) angiography to exclude the presence of LV thrombus. Under fluoroscopic guidance, an 8F NOGASTAR® mapping catheter (Cordis) was advanced to the ascending aorta, where the tip was fully deflected, and then advanced through the aortic valve into the LV. Electromechanical maps were constructed from samples of uniformly selected endocardial segments (ideally, 3 points in each of 17 segments). Results from linear local shortening and unipolar voltage were used to identify the viable ischemic myocardium and to target the injections. Each EMM was displayed in a 17-segment AHA bull's eye.[18]

Transendocardial Delivery of Cells or Saline

Each pig received a total of 13 injections. Each 0.2-mL injection of BMMNCs was performed by using the MyoStar® injection catheter (Cordis), according to the following criteria: 1) perpendicular position of the catheter to the LV wall; 2) excellent loop stability (<4 mm); 3) underlying voltage >5 mV; and 4) presence of a premature ventricular contraction on extension of the needle into the myocardium. The injection catheter comprises a 27G needle housed in an 8F catheter that is able to evaluate endocardial voltage and catheter endocardial contact. The use of EMM-guided injections enables the targeting of viable ischemic myocardium in the LCx distribution.

Tissue Preparation and Histopathology

At day 60, the pigs were humanely killed, and their hearts were rapidly excised and weighed. The pericardial sacs were examined for signs of effusion and inflammation. The hearts were sliced into 1-cm sections (breadloaf technique), photographed on each side, and then either frozen and stored at –80 °C or fixed in formalin. Each slice was subsequently divided into 4 to 6 smaller segments for correlation with the EMM segments.

Formalin-fixed/paraffin-embedded samples (average of 16 samples per heart) were stained with hematoxylin and eosin (H&E) and Masson trichrome stains for histologic examination under light microscopy. In H&E sections, the magnitude of inflammation and scarring and the presence of foreign tissue and abnormal growth were evaluated, and the entire thickness of the ventricular wall was noted. Fibrosis was quantified on trichrome-stained slides. Capillary density was evaluated by immunohistochemical staining against von Willebrand factor (Dako; Carpinteria, Calif) and was quantified with the use of Olympus Microsuite™ FIVE Imaging Software (Olympus America Inc.; Center Valley, Pa) mounted on an Olympus BX61® microscope.

Histopathologic analysis of the hearts included 1) an overall comparison of fibrosis and capillary density within groups and 2) a description of a segmental dosing threshold that was based on the mean cell concentration per segment (see Segmental Dosing Threshold).

Statistical Analysis

We used the NCSS statistical software package (Kaysville, Utah) for data analyses. Continuous variables were described as average ± SD. Comparisons between groups were performed by use of the Student *t* test for parametric data and the Wilcoxon rank sum test for nonparametric data. $P < 0.05$ was considered statistically significant.

Results

No major sequelae (cardiac perforation, cardiac tamponade, or malignant arrhythmia) were seen in any pig during either EMM or transendocardial injection.

Electrocardiography and Implantable Loop Recorder

No significant changes were noted in the PR, QRS, or QTc intervals throughout the study (Table I). No arrhythmia or conduction block occurred during the mapping or injection procedures.

Implantable loop recorders were interrogated periodically (every 4–7 days) for possible cell-therapy–related cardiac arrhythmia. During the first 30 days after ameroid placement, 2 episodes of nonsustained ventricular tachycardia occurred in 2 pigs (1 each in the control and the 50×10^6 million BMMNC groups). No increase in the frequency of arrhythmias was noted after injections. An isolated episode of nonsustained ventricular tachycardia was observed in a control animal 21 days after injection, and 2 episodes were seen in pigs in the 200×10^6 BMMNC group. Importantly, no malignant ventricular arrhythmia was recorded during the study.

Histopathologic Analyses

There was no evidence of pericardial effusion, myocardial inflammation, foreign tissue, or abnormal growth.

Fibrosis and Capillary Density within Groups

Figure 1A shows the comparison of fibrosis among the 4 groups. The absolute amount of fibrosis was higher

TABLE I. Electrocardiographic Analysis at 3 Time Points

Group and Variable	Baseline	30-Day (Injection)	60-Day (Euthanasia)	*P* Value
Control				
PR interval, ms	114 ± 8.33	138 ± 30.9	120 ± 3.27	0.146
QRS interval, ms	62 ± 6.93	64 ± 9.8	68 ± 8.64	0.616
QTc interval, ms	467.5 ± 21.4	450 ± 9.6	468.81 ± 23.2	0.358
50×10^6 cells				
PR interval, ms	110 ± 15.14	115 ± 7.57	130 ± 22.7	0.256
QRS interval, ms	67 ± 8.87	69 ± 6	80 ± 4	0.084
QTc interval, ms	450.2 ± 9.5	468.25 ± 16.6	493.75 ± 88.7	0.155
100×10^6 cells				
PR interval, ms	104 ± 5.66	121 ± 12.81	127 ± 16.12	0.062
QRS interval, ms	63 ± 5.03	67 ± 8.87	65 ± 2	0.655
QTc interval, ms	449 ± 59.9	473.25 ± 24.9	493.25 ± 36.3	0.389
200×10^6 cells				
PR interval, ms	108 ± 5.66	122 ± 10.58	126 ± 12.44	0.225
QRS interval, ms	60 ± 0	62 ± 2.31	66 ± 4	0.11
QTc interval, ms	476 ± 16.9	517.5 ± 81.5	452.25 ± 74.1	0.425

Results are presented as the average ± SD. $P < 0.05$ was considered statistically significant.

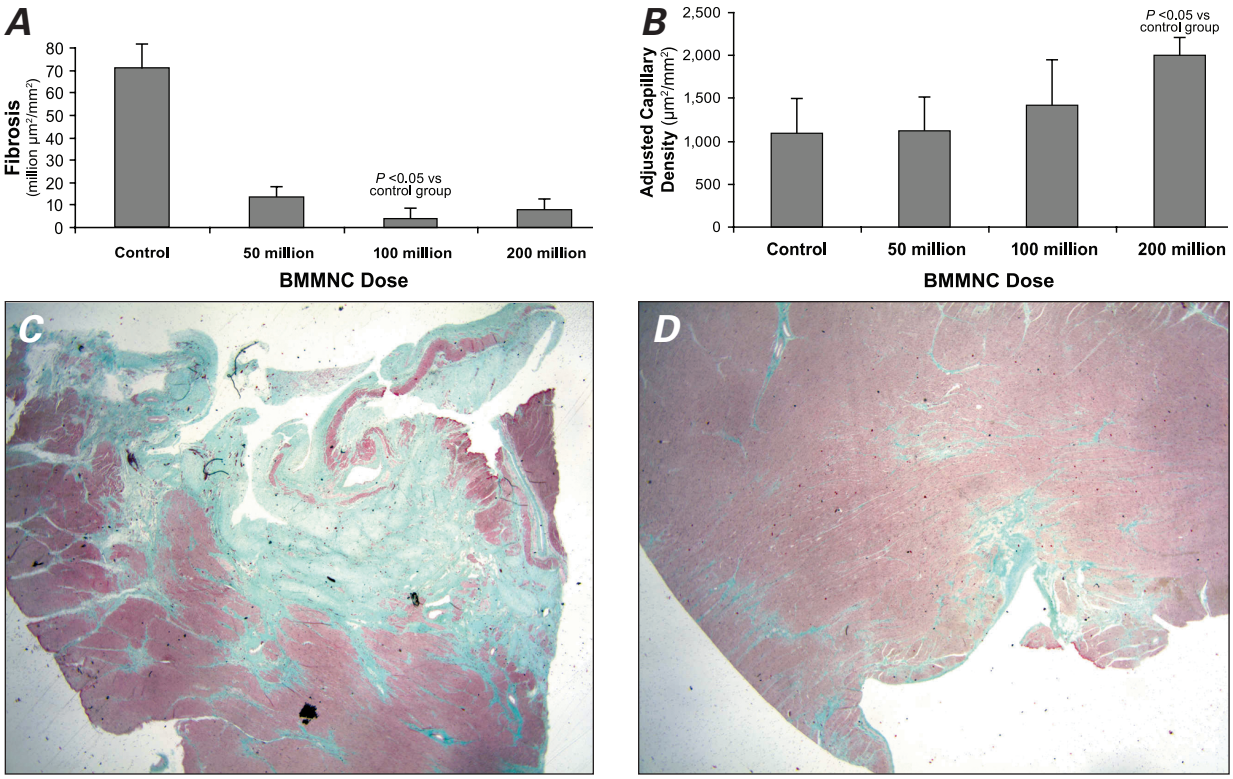

***Fig. 1 A**) Volume of fibrosis in the control group and in the 3 groups that received cell doses. **B**) Adjusted capillary density in the injured segment (adjusted to the volume of fibrosis by using the ratio between the absolute capillary density divided by the volume of scar tissue). Injected areas of **C**) a control pig (Masson trichrome stain, orig. ×1.25) and **D**) a pig treated with 100 × 10^6 BMMNC (Masson trichrome stain, orig. ×4) show reduced fibrosis after BMMNC injection.*

BMMNC = bone marrow mononuclear cell

in the control group than in any treatment group, but the difference was statistically significant only in the comparison between control pigs and those in the 100 × 10^6 BMMNC group. Capillary density was similar in the control and the 50 × 10^6 BMMNC groups and increased progressively with cell dose (Fig. 1B), to the point where we found a statistically significant difference between the 200 × 10^6 BMMNC group and the control group (Fig. 1B). Improved healing after BMMNC injection is shown in Figures 1C and 1D.

Segmental Dosing Threshold

The mean cell concentration per segment, considering all 3 cell-treated groups, was 22.8 ± 17.1 million BMMNCs. On the basis of the mean cell concentration per segment, we evaluated the local effects of cell concentration on fibrosis and capillary density by dividing the treated segments into high-dose (>20 million cells) and low-dose (<20 million) groups. Significantly less fibrosis was seen in all cell-treated segments (both high- and low-dose groups) when compared with control segments (Fig. 2A). In evaluating capillary density, this difference was more evident in the comparison of control and high-dose cell-treated concentration segments (Fig. 2B). Consequently, the segments that received the highest cell concentration had the highest capillary density and the least amount of fibrosis.

Discussion

This study provides evidence that higher doses of BMMNCs administered via transendocardial injection are safe and feasible in the setting of chronic myocardial ischemia. Higher cell doses were not associated with electrical instability or ventricular arrhythmias, and histopathologic study showed no inflammation at 30-day follow-up examination. Furthermore, our results suggest the presence of a segmental dosing threshold.

Previous studies have not reported an increased incidence of myocardial arrhythmias after BMMNC injections in the setting of chronic myocardial ischemia. However, Fukushima and colleagues[15] have challenged this belief; in their postmyocardial infarction model of heart failure in rats, they ligated the left coronary artery and, 21 days later, administered 10 × 10^6 BMMNCs via direct intramyocardial injection or through a retrograde intracoronary route. Rats that received cells via intramyocardial delivery had a higher incidence of electrical instability (ventricular arrhythmias) in the first 7 to 14 days after cell delivery than did rats that underwent in-

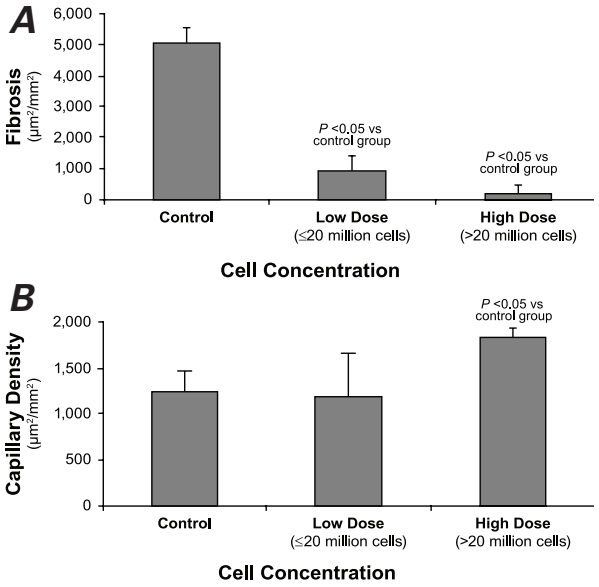

Fig. 2 *Segmental analyses compare* ***A****) fibrosis and* ***B****) capillary density with cell concentration.*

tracoronary delivery. Findings from the present study do not support the hypothesis that intramyocardial injection of BMMNCs induces arrhythmias. One possible explanation for the arrhythmogenicity seen in the study by Fukushima and colleagues is their use of only 2 nontargeted cell injections, which requires a high volume for each injection. In contrast, the delivery approach in the present study is different and involves targeting viable myocardium with the use of smaller volumes per injection (0.2 mL) and multiple injection sites. All clinical trials to date in which multiple, small-volume injections of BMMNCs have been used to treat ischemic heart disease have led to findings similar to those shown in the present study.

Important clinical studies within the context of AMI have provided insight into the dosing aspect of BMMNCs delivered through the intracoronary route. Clinical studies[18,19] have shown that the number of cells delivered via the intracoronary route after AMI did not correlate with functional improvement in perfusion or contractile end points. In contrast, Meluzin and colleagues[14] showed that intracoronary-delivered BMMNCs improved regional myocardial function in a dose-dependent manner.

Catheter-based injections of bone marrow stem cells have been shown to improve capillary density and reduce the amount of fibrosis in large-animal models of myocardial ischemia.[20,21] Protocols for cell injections have used doses ranging from 1×10^5 up to 1×10^8 bone-marrow–derived stem cells. The ideal dose of BMMNCs for transendocardial delivery in the setting of chronic myocardial ischemia has not been established. Our findings suggest the importance of cell dosing in the treatment of chronic myocardial ischemia. In our study, the amount of fibrosis was significantly lower than control levels only in the medium-dose group (100×10^6 BMMNCs); the high-dose group (200×10^6) had an intermediate amount of fibrosis, falling between the low-dose (50×10^6) and medium-dose (100×10^6) groups. The magnitude of reduction of fibrosis in the 3 groups, when compared with the control group, was relatively uniform, suggesting that higher doses of cells have little value in reducing fibrosis. This point was confirmed by the segmental analysis. Both high- and low-dose segments had statistically significant reductions in the amount of fibrosis.

Capillary density progressively increased with cell dose in our study, but the small number of animals might have prevented the higher absolute capillary density from reaching statistical significance. The absolute capillary density was the same for the low-dose cell group and the control group; the higher absolute capillary density was reached with the medium dose of 100×10^6 cells, which suggested that a "dosing threshold" might exist. A dose-dependent effect was suggested in global capillary density, because the 200×10^6 cell group had higher capillary-density numbers than did the 100×10^6 cell group, which had higher numbers than did the 50×10^6 cell group. The dose-threshold effect is further suggested by higher segmental capillary density only in the high-dose segments (greater than 20×10^6 BMMNCs). In a recent preclinical study, Dixon and colleagues[22] examined the effects of 4 different concentrations of mesenchymal precursor cells delivered after myocardial infarction in sheep. They found a threshold of efficacy when transplanted cells were injected in the border zone of the infarct; sheep treated with the lowest dose appeared to have a more advantageous healing environment. Our findings also suggest a threshold effect with the use of a different cell type (BMMNC) in a model of chronic ischemia.

The finding of a possible segmental dosing threshold might indicate that a different approach to cell dosing should be considered: dosing in proportion to the area at risk. In fact, this approach parallels our current understanding of pharmacology, in which drug dosages are derived on the basis of the total volume of distribution. Similarly, the optimal cell doses might be based on the number of ischemic segments. This approach could influence future studies of cell injections in chronic ischemia by changing the delivery approach to one in which injections sites are carefully chosen and occasionally clustered, targeting the viable ischemic myocardium, to optimize the segmental benefit of cell therapy.

Limitations. The major limitation of the present study is the small number of animals, which prevents the use of rigorous statistical tests of comparisons, such as an analysis of variance, and therefore limits conclusions about efficacy. The differences between the groups

might have occurred by chance. In addition, there are inherent limitations in the ameroid constrictor model in that the size of the constricted artery affects the area of ischemia, so some animals received more cell injections. However, this is one of the first large-animal, preclinical studies of the important issue of cell dosing in this nascent field. Moreover, the segmental analysis provides new insight into cell dosing.

Summary. The results of this preclinical study support the idea of clustering the injection sites to areas of viable ischemic myocardium, which provides higher local concentrations of cells in the segments exposed to more intense injury. The best results were seen in segments that received more than 20×10^6 BMMNCs. A segmental dosing approach with a dose that is proportional to the area at risk is suggested. These findings influence future protocols for transendocardial injections of cells and require further evaluation in preclinical and clinical studies.

References

1. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 2003;361(9351):47-9.
2. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Silva GV, et al. Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. Circulation 2004;110(11 Suppl 1):II213-8.
3. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation 2003;107(18):2294-302.
4. Bartunek J, Vanderheyden M, Vandekerckhove B, Mansour S, De Bruyne B, De Bondt P, et al. Intracoronary injection of CD133-positive enriched bone marrow progenitor cells promotes cardiac recovery after recent myocardial infarction: feasibility and safety. Circulation 2005;112(9 Suppl):I178-83.
5. Assmus B, Schachinger V, Teupe C, Britten M, Lehmann R, Dobert N, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002;106(24):3009-17.
6. Schachinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI trial. J Am Coll Cardiol 2004;44(8):1690-9.
7. Beeres SL, Bax JJ, Zeppenfeld K, Dibbets-Schneider P, Stokkel MP, Fibbe WE, et al. Feasibility of trans-endocardial cell transplantation in chronic ischaemia. Heart 2007;93(1):113-4.
8. Beeres SL, Bax JJ, Dibbets P, Stokkel MP, Zeppenfeld K, Fibbe WE, et al. Effect of intramyocardial injection of autologous bone marrow-derived mononuclear cells on perfusion, function, and viability in patients with drug-refractory chronic ischemia. J Nucl Med 2006;47(4):574-80.
9. Tse HF, Thambar S, Kwong YL, Rowlings P, Bellamy G, McCrohon J, et al. Safety of catheter-based intramyocardial autologous bone marrow cells implantation for therapeutic angiogenesis. Am J Cardiol 2006;98(1):60-2.
10. Fuchs S, Hendel RC, Baim DS, Moses JW, Pierre A, Laham RJ, et al. Comparison of endocardial electromechanical mapping with radionuclide perfusion imaging to assess myocardial viability and severity of myocardial ischemia in angina pectoris. Am J Cardiol 2001;87(7):874-80.
11. Perin EC, Silva GV, Sarmento-Leite R, Sousa AL, Howell M, Muthupillai R, et al. Assessing myocardial viability and infarct transmurality with left ventricular electromechanical mapping in patients with stable coronary artery disease: validation by delayed-enhancement magnetic resonance imaging. Circulation 2002;106(8):957-61.
12. Poppas A, Sheehan FH, Reisman M, Harms V, Kornowski R. Validation of viability assessment by electromechanical mapping by three-dimensional reconstruction with dobutamine stress echocardiography in patients with coronary artery disease. Am J Cardiol 2004;93(9):1097-101.
13. Hou D, Youssef EA, Brinton TJ, Zhang P, Rogers P, Price ET, et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation 2005;112(9 Suppl):I150-6.
14. Meluzin J, Mayer J, Groch L, Janousek S, Hornacek I, Hlinomaz O, et al. Autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction: the effect of the dose of transplanted cells on myocardial function. Am Heart J 2006;152(5):975.e9-15.
15. Fukushima S, Varela-Carver A, Coppen SR, Yamahara K, Felkin LE, Lee J, et al. Direct intramyocardial but not intracoronary injection of bone marrow cells induces ventricular arrhythmias in a rat chronic ischemic heart failure model. Circulation 2007;115(17):2254-61.
16. Perin EC, Silva GV, Sarmento-Leite R, Vaughn WK, Fish RD, Ferguson JJ 3rd. Left ventricular electromechanical mapping: preliminary evidence of electromechanical changes after successful coronary intervention. Am Heart J 2002;144(4):693-701.
17. Sarmento-Leite R, Silva GV, Dohman HF, Rocha RM, Dohman HJ, de Mattos ND, et al. Comparison of left ventricular electromechanical mapping and left ventricular angiography: defining practical standards for analysis of NOGA maps. Tex Heart Inst J 2003;30(1):19-26.
18. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med 2006;355(12):1199-209.
19. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, et al. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) trial. Circulation 2006;113(10):1287-94.
20. Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL, et al. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. J Mol Cell Cardiol 2008;44(3):486-95.
21. Wisenberg G, Lekx K, Zabel P, Kong H, Mann R, Zeman PR, et al. Cell tracking and therapy evaluation of bone marrow monocytes and stromal cells using SPECT and CMR in a canine model of myocardial infarction. J Cardiovasc Magn Reson 2009;11:11.
22. Dixon JA, Gorman RC, Stroud RE, Bouges S, Hirotsugu H, Gorman JH 3rd, et al. Mesenchymal cell transplantation and myocardial remodeling after myocardial infarction. Circulation 2009;120(11 Suppl):S220-9.