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Alberto Farinetti*, Roberto Lonardi*, Alberto Barbieri*, Enrico Giuliani*, Sonia Pennella**, Moreno Pozza*, Massimo Saviano*, Antonio Manenti*, Anna Vittoria Mattioli***

*Department of Surgery, University of Modena and Reggio Emilia, Modena, Italy

**Istituto Nazionale di Ricerche Cardiovascolari, U.O. Modena, Italy

***Department of Biomedical Science, University of Modena and Reggio Emilia, Modena, Italy

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AIM: *The aim of the study was to evaluate the surgical approach to intramyocardial (i.m.) injection of Bone Marrow Stem Cells (BMSCs) in a pre-clinical model and its complications.*

MATERIAL OF STUDY: *In New Zealand rabbits an ischemia reperfusion injury lasting 20 min was induced by temporary ligation of anterior descending coronary artery during cardiac surgical procedure. Homologous BMSCs were isolated from the posterior iliac crest, cultured and re-suspended for injection. BMSC were injected at the peri-infarcted area and side effects were evaluated. A control group with myocardial infarction was treated with i.m. injections of saline, to evaluate possible side effects of injection. Comparison of ventricular premature contractions (VPC), ventricular tachycardia and ventricular fibrillation were recorded during surgery and after 7 and 21 days.*

RESULTS: *Seven rabbits developed intractable ventricular fibrillation during the experimental protocol, three during coronary ligation but before cell injections and four following i.m. injections. At day 7, hourly PVC were more frequent in the groups of animals that received i.m. injections of BMSCs (132 ± 19 beats) compared to saline injections. (54 ± 14).*

CONCLUSIONS: *Intramyocardial injections of BMSCs induced an electrical instability as shown by a high number of PVC as compared with intramyocardial injections of saline.*

KEY WORDS: Cardiac surgery, Intramyocardial injections, Ischemia/reperfusion, Pre-Clinical model, Stem cells.

Introduction

Myocardial infarction (MI) is a life-threatening event that may cause sudden cardiac death and heart failure. Damaged myocardium after acute MI is gradually replaced by fibrotic noncontractile cells to form scar tissue.

Cell transplantation is a promising approach offering the possibility of developing a of new functional tissues to replace the damaged cardiomyocytes. However the clinical use of stem cells, i.e. autologous Bone Marrow Stem Cells (BMSCs) in acute MI provides conflicting results¹⁻³. Moreover, the concern that delivery of stem cells could cause potentially life-threatening ventricular arrhythmias has been repeatedly raised⁴. Occurrence of ventricular arrhythmias was reported in patients injected with skeletal myoblasts⁵. Menasche and coworkers suggested that failure of differentiated myotubes to express gap junction proteins resulted in electrically insulated cell clusters which predispose to reentry circuits^{5,6}.

The aim of the present study was to evaluate the surgical approach to intramyocardial (i.m.) injection of Bone Marrow Stem Cells (BMSCs) in a pre-clinical model and its complications.

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Correspondence to: Alberto Farinetti, MD, Department of Surgery, University of Modena and R.E., Via del Pozzo 71, 41124 Modena (Italy) (E-mail: alberto.farinetti@unimore.it)

Materials and Methods

Study population and surgical procedure. In 23 New Zealand rabbits, weighting 4 kg (mean 4.1 + 0.5 kg) an acute ischemia reperfusion injury was induced by ligation of left anterior descending coronary artery (LAD).

The rabbits were sedated by intramuscular (quadriceps femoris) injection of 1-2 mg/kg -1 medetomidine and 0.7-1 mg/kg -1 midazolam under animal handler direct supervision to reduce stress reaction. The marginal auricular vein was cannulated with a 22 G catheter and saline infusion was started at 1-1.5 ml/kg/min. Initial bolus of 2-3 mg/kg -1 of 1% propofol solution was administered 2 minutes prior surgical positioning and every 5 minutes to maintain anaesthesia. Thereafter a muzzle mask was positioned over nose and mouth and a bag-valve 0.5-1 ventilation system was connected to an oxygen supply. Respiratory rate was monitored by counting reservoir volume variations. A twelve lead electrocardiogram was recorded throughout the operation. A left thoracotomy was made through the fifth intercostal space and the pericardium was open. To create an anterior MI, a distal portion of the LAD was select for ligation. The selected artery was temporarily occluded and the extent of ischemia was visually and electrocardiographically assessed. After 20 minutes of ischemia the area was reperfused. A successful induction of AMI will be confirmed by elevation of the ST segment by more than 0.2 mV in leads I, II and aVL.

BM cell collection and culture. BMSCs were aspirated from posterior iliac crest, were cultured and re-suspended in saline (NaCl 0.9%) for injection at the level of peri-infarcted zone (6 injections). BMSCs were isolated by magnetic separation (MACs Miltenyi Biotec S.r.l. Calderara di Reno, Bologna, Italy).

Animals were treated as follows: Group 1: i.m. injections of BMSCs and Group 2: i.m. injection of saline (to evaluate pro- arrhythmic effect of i.m. injections).

After the surgical procedure, the chest was closed. All animals received postoperative antimicrobial therapy (cephazoline 1.0 i.m. twice/day for 3 days) and buprenorphine (0.3 mg i.m. twice/day for 3 days) for postoperative pain. An ECG recording system (Mortara Instrument, Eli 150, Italy), was localized over the neck of the animals and recorded ECG the day before surgery, (time 0), during the entire surgical procedure (time 1), and for the 7 days following surgery (time 2). A 24 hour ECG-Holter was performed at day 21 before sacrifice (time 3). The following parameters were evaluated: hourly number of supraventricular and ventricular premature contractions (VPCs), ventricular tachycardia (VT), ventricular fibrillation (VF).

Animals were monitored for the next 21 days. All animals received care in compliance with the European Convention on Animal Care. Animal care was provided by the same trained operator in order to reduce stress. The study was approved by the Research Animal Care and Use Committee of the University of Modena and Reggio Emilia (Italy) (prot. 25, 18.03.2008 – prot. 83, 25.09.2009).

STATISTICAL ANALYSIS

SPSS, V.14.0.1 (SPSS Inc, Chicago, Ill), was used for statistical analysis. Statistical analysis of data within and between groups was performed with one-way and two-way analysis of variance. Changes were considered statistically significant when the P value was less than 0.05. All data are expressed as mean + SEM.

Results

Average duration of surgical time was 25 minutes from incision to suture (range 20-30 minutes). Of the initial 23 rabbits, seven developed intractable VF during the experimental protocol, three during coronary ligation but before cell injections and four following i.m. injections (2 from group 1 and 2 from group 2; p=n.s.). All these rabbits showed a small pericardial effusion after opening the pericardium, however no histological changes were found. The remaining 16 rabbits were evaluated for 20 to 30 days before sacrifice (Table I).

During ischemia we registered three episodes of reversible ventricular tachycardia. At day 7 we reported a higher

TABLE I - Baseline Characteristics of the three groups of animals.

	BMSC i.m. injection	BMSC i.v. injection	Saline i.m.
Weight (Kg)	4.2 ± 0.6	4.1 ± 0.4	4.15 ± 0.6
Female/male	4/4	5/3	5/3
Heart rate (b/min)	200 ± 16	201 ± 10	196 ± 18
QTc (ms)	422 ± 16	419 ± 20	420 ± 25
VPC at day 0 before surgery	11 ± 4	13 ± 6	14 ± 5

Legend: QTc: corrected QT interval; VPC: ventricular premature contraction; SVPC: supra-ventricular premature contraction.

TABLE II - Parameters from ECG analysis.

	BMSC i.m. injection	BMSC i.v. injection	Saline i.m.
HR at time 0 (b/min)	200 ± 16	201 ± 10	196 ± 18
HR during ischemia (b/min)	186 ± 20	199 ± 25	184 ± 23
HR at day 7 (b/min)	202 ± 21	210 ± 21	208 ± 14
HR at day 21 (b/min)	200 ± 24	198 ± 22	203 ± 19
VPC at time 0 (hourly)	11 ± 4	13 ± 6	14 ± 5
VPC at day 7 (hourly)	132 ± 19**	34 ± 9**	54 ± 14**
VPC at day21 (hourly)	96 ± 23**	52 ± 19**	25 ± 20
QTc at time 0 (ms)	418 ± 16	416 ± 20	415 ± 25
QTc during ischemia (ms)	432 ± 21*	427 ± 23*	434 ± 28*

Legend: Time 0= day before surgery; *p<0.05 vs time 0 (pre-ischemia); **p<0.001 vs time 0 (pre-ischemia).

number of PVC in animals treated with i.m. injections of BMSCs compared with group 2 treated with i.m. saline (130 ± 21 versus 50 ± 10 ; $p < 0.01$) (Table II). Macroscopically, ligation of the LAD resulted in a relatively small, transmural infarct in the apex of the left ventricle. Histopathology showed no chronic inflammatory cell infiltrates or evidence of rejection. However several foci of moderate fibrosis were present on the endomyocardial surface across all groups.

Discussion

The present study provided information on side effects of i.m. injection of BMSCs. We found that animals receiving i.m. BMSCs developed more VPC compared with rabbits receiving i.m. saline injections, suggesting a potential role of cells transplantation in the mechanisms of arrhythmias.

Rabbits are widely used as animal models for various biological and medical applications. The feasibility of surgical procedures is dependent from the effectiveness of the anesthetic approach. Intravenous anesthesia requires just a peripheral venous catheter and a muzzle mask to be performed, without inducing a relevant respiratory depression or bradycardia. Infact cardiac output and consequently organ perfusion is dependent on HR, so any marked reduction can impact negatively on outcome and potentially lead to cardiac arrest. Rabbits behavior seems to affect their reaction to anesthesia as those who manifest aggressiveness prior to sedation will likely develop resistance towards pharmaceutical treatment requiring higher propofol doses with the risk of respiratory depression; the reason for this anomalous reaction is unknown. However, it is well known, that rabbits have a raised adrenergic tone which could to explain why stress impact on response to anesthesia.

An important issue of regenerative therapy in acute myocardial infarction is that therapeutic efficacy and arrhythmia occurrence induced by cell injection into the post-MI heart may be affected by the cell delivery route. The i.m. route can easily deliver cells selectively into target areas by direct visualization of the infarct scar; however, this method causes mechanical injury and subsequent acute inflammation. In addition, cells grafted via the intramyocardial route are predisposed to form islet like cell clusters that are isolated and not coupled with the host myocardium. Such local heterogeneity in the myocardium is considered a potential source of arrhythmias^{7,8}. A previous study performed in pig, showed that i.m. transplantation is superior to the intra-coronary transplantation because of the high rate of cell homing at the infarction site. When the i.m. route is used, most of the cells are retained in the target area; the efficacy of the intra coronary route is only 12.3% of that of the i.m. three weeks after infarction⁹.

The present study was designed to evaluate side effects,

i.e. arrhythmias of cells injection. During the acute phase of myocardial infarction the intramyocardial administration of cells induced a high number of ventricular ectopic beats. However intramyocardial administration of saline, also, induced a high number of PVC suggesting a pro-arrhythmic effect of the direct injection. Moreover data registered after 7 days of MI induction showed a greater number of PVC in animals treated with i.m. injections of BMSCs compared with animals treated with saline suggesting a pro-arrhythmic effect strictly related to BMSCs. During the acute phase, intramyocardial injections could act as trigger for PVC and VF independently from the presence or absence of cells within saline solution. The mechanisms could be related to mechanical injury done by needle.

On contrary during the sub acute phase of MI, PVC could be related to an incomplete differentiation of cells toward cardiomyocytes. Injections of BMSCs stimulate growth factors and cytokines which cannot be synthesized by from cardiac myocytes that have been lost during infarction. Such paracrine and autocrine factors are involved in many aspects of cardiac repair¹⁰⁻¹². These results may be referred to the beneficial effect of BMSCs since it is known that healing may be associated with an increased number of PVC. It is also well known that the reason of arrhythmias in MI may be quite different dependent on the time after the acute event; during the acute phase ischemic triggered arrhythmias may prevail, whereas during the late phase scarring and remodelling are main factors for the occurrence of PVC.

Moreover the differentiation of BMSCs into cardiomyocytes is not always associated with cell-cell communication that in the myocardium is mediated by many different factors, i.e. gap-junction mediated cells contacts, cell-matrix interactions, and signalling through adhesion molecules. It is possible that the initial differentiation of cells lacks in cell-cell communication. This finding suggested that BMSCs injections could be associated with changes in cardiac electrophysiological properties. The adult human heart contains a great heterogeneity of distinct functional types of cardiomyocytes and we need to define the factors that favour differentiation to particular cardiomyocytes cell lineages¹³⁻¹⁵. The relative immaturity of cells used for cell transplantation may increase the risk of action potential mismatch with the adult host cardiomyocytes and that of persistent automaticity.

Conclusions

Intramyocardial injections of BMSCs induced an electrical instability as shown by a high number of PVC as compared with intramyocardial injections of saline. However, these preliminary data need to be confirmed by further studies evaluating a longer follow-up before translating information to clinical studies. Furthermore, there is evidence that a late post operative atrial fibril-

lation has a high incidence after cardiac surgery and correlates with the risk of heart failure with consequent negative effects on morbidity¹⁶; we cannot exclude that arrhythmic episodes may affect rabbits even after discharge from cardiac surgery¹⁷.

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Riassunto

SCOPO: Lo scopo dello studio è stato valutare l'approccio chirurgico e le sue complicanze all'iniezione intramiocardica di Cellule Staminali da Midollo Osseo in un modello preclinico.

MATERIALI: In conigli New Zealand è stato indotto un danno da ischemia e riperfusione della durata di 20 min tramite una temporanea legatura dell'arteria coronaria anteriore durante procedura di chirurgia cardiaca. Cellule Staminali da Midollo Osseo omologhe erano state isolate dalla cresta iliaca posteriore, coltivate e risospese per iniezione. Tali cellule sono poi state iniettate nell'area perinfartuata e sono stati valutati gli effetti locali. Un gruppo di controllo con infarto miocardico è stato trattato con iniezione intramuscolare di soluzione salina, per valutare possibili effetti locali indotti dall'iniezione. Sono stati registrati e confrontati il numero di battiti prematuri ventricolari e sopraventricolari, episodi di tachicardia ventricolare e fibrillazione ventricolare durante l'intervento chirurgico e dopo 7 e 21 giorni nei due gruppi.

RISULTATI: Sette conigli hanno sviluppato fibrillazione ventricolare irreversibile durante il protocollo sperimentale, tre durante la legatura coronarica ma prima dell'iniezione cellulare e 4 dopo l'iniezione intramuscolare.

Al settimo giorno i battiti prematuri ventricolari giornalieri erano più frequenti nei gruppi di animali che avevano ricevuto iniezione intramuscolare di Cellule Staminali da Midollo Osseo (132 ± 19 beats) rispetto a quelli che avevano ricevuto soluzione salina (54 ± 14).

CONCLUSIONI: L'iniezione intramiocardica di Cellule Staminali da Midollo Osseo ha indotto un'instabilità elettrica come mostrato da un alto numero di battiti prematuri ventricolari rispetto all'iniezione intramiocardica di soluzione salina suggerendo un'effetto proaritmico diretto delle cellule.

References

1. Strauer BE, Steinhoff G: *10 Years of intracoronary and intramyocardial bone marrow stem cell therapy of the heart: From the methodological origin to clinical practice.* J Am Coll Cardiol, 2011; 58:1095-104

2. Tendera M, Wojakowski W, Ruzyłło W, et al.: *REGENT Investigators. Intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: Results of randomized, multicentre Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) Trial.* Eur Heart J, 2009; 30:1313-321.

3. Schächinger V, Erbs S, Elsässer A, et al.: *Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction.* N Engl J Med, 2006; 355:1210-221.

4. Menasché P: *Stem Cell Therapy for Heart Failure. Are Arrhythmias a Real Safety Concern?* Circulation, 2009; 119:2735-740.

5. Menasché P, Alfieri O, Janssens S, et al.: *The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) Trial. First randomized placebo-controlled study of myoblast transplantation.* Circulation, 2008; 117:1189-200.

6. Menasché P: *Cardiac cell therapy: Lessons from clinical trials.* J Mol Cell Cardiol, (2010), doi:10.1016/j.yjmcc.2010.06.010.

7. Podesser B, Wollenek G, Seitelberger R, et al.: *Epicardial branches of the coronary arteries and their distribution in the rabbit heart: The rabbit heart as a model of regional ischemia.* The Anatomical Record, 1997; 247:521-27.

8. Brunskill SJ, Hyde CJ, Doree CJ, et al.: *Route of delivery and baseline left ventricular ejection fraction, key factors of bone-marrow-derived cell therapy for ischaemic heart disease.* Eur J Heart Fail, 2009; 11:887-96.

9. Makela J, Anttila V, Ylitalo K, et al.: *Acute homing of bone marrow-derived mononuclear cells in intramyocardial vs. intracoronary transplantation.* Scand Cardiovasc Journ, 2009; 43:366-73.

10. Tirziu D, Giordano FJ, Simons M: *Cell Communications in the heart.* Circulation, 2010; 122:928-37.

11. Cho HJ, Lee N, Lee JY, et al.: *Role of host tissues for sustained humoral effects after endothelial progenitor cell transplantation into the ischemic heart.* J Exp Med, 2007; 204:3257-269.

12. Caplan AI, Dennis JE: *Mesenchymal stem cells as trophic mediators.* J Cell Biochem, 2006; 98:1076-84.

13. Redwood SR, Taggart PI, Sutton PM, et al.: *Effect of magnesium on the monophasic action potential during early ischemia in the in vivo human heart.* J Am Coll Cardiol, 1996; 28:1765-769.

14. de Bruyne MC, Hoes AW, Kors JA, et al.: *QTc dispersion predicts cardiac mortality in the elderly: The Rotterdam Study.* Circulation, 1998; 97:467-72.

15. He JQ, Ma Y, Lee Y, et al.: *Human embryonic stem cells develop into multiple types of cardiac myocytes action potential characterization.* Circ Res, 2003; 93:32-39.

16. Capucci A, Guerra F: *Late postoperative atrial fibrillation: minor surgery complication or major cardiovascular issue?* J Cardiovasc Med 2011; 12:381-82.

17. Ambrosetti M, Tramarin R, Griffo R, et al.: *Late postoperative atrial fibrillation after cardiac surgery: a national survey within the cardiac rehabilitation setting.* J Cardiovasc Med, 2011; 12:390-95.