We should also consider the difficulties that the operator has to overcome during the implantation of this stent. Passage of the MGS through tortuous, calcified or small diameter vessels is very challenging, if not impossible. The few cases, where we failed to advance the stent through the lesion, were shepherds crook type of RCA anatomy. Another important issue is the compromising of side branches by the presence of polymer mesh sleeve on its external surface. In addition, there are no data about its behavior in high-thrombotic volume, especially when no other EPD were used. In our study, there were a few cases with thrombus load grade five, where M-Guard was used with the aspiration catheter and the effectiveness of their combination was impressive, resulting in TIMI 3 flow with satisfactory blush score.

The limitations of our study concern the relatively small number of patients, taking mostly into consideration the subgroup of patients from the MGS group that TA was not performed. Another aspect is also the lack of a randomized design.

In conclusion, in the setting of pPCI, MGS proved to be effective and safe with low TVR and low thrombosis rates at 1 and 12 months, considering the high risk group of STEMI patients. In addition, final TIMI flow and MBG are higher and the incidence of no-reflow is less. That benefit from its use is independent from the performance of TA in pPCI.

The authors report no relationships that could be construed as a conflict of interest.

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http://dx.doi.org/10.1016/j.ijcard.2014.07.025

Intracoronary mesenchymal stem cell transplantation in patients with ischemic cardiomyopathy

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ARTICLE INFO

Article history:
Received 14 April 2014
Accepted 5 July 2014
Available online 11 July 2014

Keywords:
Mesenchymal stem cells
Myocardial infarction
Gene expression
Cell growth

Dear Editor,

Mesenchymal stem cells have unique ability to detect the injured tissue and heal it. This is the main reason why they are commonly used in regenerative therapy. Their protective effect is mediated by a paracrine way, cell-to-cell contact and other unknown mechanisms [1]. The patient treatment after myocardial infarction by autologous MSC results in an increased ejection fraction. It is safe and feasible [2]. The similar result has been proven by intramyocardial stem cell injection in patients with ischemic cardiomyopathy. The result was a partial revitalization of the infarct area and a decrease of the end-systolic and end-diastolic volume in one year after the cell administration [3]. In the present pilot study, we have shown the difference in the MSC gene expression profile from healthy donors versus patients after myocardial infarction. Our aim was to gain a better understanding of the therapeutic effect of autologous bone marrow-derived mesenchymal stem cells in patients with heart failure.

Ten patients with advanced chronic heart failure as a result of ischemic cardiomyopathy were included in the study. All of them were males with mean age of 50 years (range 43–59). All had well documented history of anterior wall acute myocardial infarction (AMI). AMI occurred at median of 38 months prior to the entry to the study. In two patients reperfusion for AMI was performed using balloon angioplasty, in four patients with thrombolysis and in four patients no reperfusion was used. In four patients percutaneous coronary intervention with stenting (because of chronic hemodynamically significant coronary artery stenosis) was performed more than 4 months before the procedure. One patient was five years after CAGB. In two patients an implantable cardioverter defibrillator was implanted 3 and 4 years earlier. In five patients coronary angiography before the MSC injection showed nonsignificant stenoses in infarct related artery (LAD), the other five patients had coronary lesions on small LAD branches (not suitable for any intervention). Local institutional ethic committee approved the study protocol in November 2003. Written informed consent was obtained from all patients.

Thirty milliliters of bone marrow blood was obtained from the posterior iliac crest under local anesthesia by a standard technique from the patients as well as from healthy donors. (The aim of MSC isolation from the healthy donors was to treat the recipient GvHD after HLA identical sibling stem cell transplantation).

The MSC were isolated as described previously [4]. The obtained bone marrow blood was diluted with 70 ml of PBS (phosphate buffered saline) and centrifuged 900 ×*g* for 10 min at 20 °C. The sediments were resuspended in 30 ml PBS, overlaid (15 ml each) over 35 ml of Percoll solution (density 1073 g/ml) and centrifuged 900 ×*g* for 30 min at 20 °C. Mononuclear cells (MNC) at the interphase were removed, diluted with PBS to 50 ml and centrifuged 460 ×*g* for 10 min at 20 °C. The sediments were resuspended in PBS. The MNC were

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Table 1
The patient survival.

Patient No	Tx (date)	Status in 03/14	Survival (months)
1	02/04	Alive	121+
2	02/04	Dead	119
3	03/04	Alive	120+
4	04/04	Dead	37
5	08/04	Dead	71
6	10/04	Alive	113+
7	10/04	Dead	9
8	11/04	Alive	112+
9	04/05	Dead	7
10	04/05	Dead	101

added to 35 ml DMEM low glucose which contained 10% FBS and incubated at 37 °C in 5% CO2. After 48 h the nonadherent cells were removed and the adherent cells were incubated in the same medium as above at 37 °C in 5% CO2. The medium was changed every 72 h until the cells reached 90–100% confluence. Adherent cells were detached after 5–7 min treatment with 0.05% trypsine-EDTA, centrifuged, washed and resuspended in 12 ml PBS supplemented with 2% human serum albumin. The MSC fulfilled the criteria provided by The International Society for Cellular Therapy [5]. Briefly, MSC are defined by their plastic-adherent properties under standard culture conditions, by their ability to differentiate into osteocytes, adipocytes and chondrocytes in vitro under specific stimulus and by positive (CD105, CD73, and CD90) or negative (CD45, CD34, CD14 and HLA-DR) expression of specific surface markers.

The freshly prepared detached MSC in PBS and 2% human serum albumin were injected in the LAD artery in a median time of 43 min. Standard coronary catheterization was performed using an over-the-wire balloon catheter (Concerto, OCCAM). The balloon was inflated in proximal part of LAD. During the first 3 minute lasting inflation no cells were injected. Thereafter, the suspension of MSC was injected in four 3 ml boluses during low pressure coronary artery occlusions lasting 3 min. Intermittent protocol of cell injections with balloon occlusion of LAD was performed to prevent back-flow, trigger ischemia and prolong contact time for cell migration. After the procedure patients were monitored at the coronary care unit up to 24 h. Before and then 2, 6, 12 and 24 h after the procedure the ECG, the serum concentration of MB creatine kinase (CK-MB), troponin T (Trop-T), brain natriuretic peptide (BNP) and C reactive (CRP) protein were measured. BNP and CRP were estimated before discharge. ECG Holter monitoring was performed at baseline before discharge and 14 days after. All patients tolerated MSC injection well and were discharged from the hospital 3–4 days after the procedure. No clinical, ECG signs of acute myocardial lesion were observed. No significant dysrhythmia or increase in ventricular ectopic activity was noted during monitoring after the procedure or at Holter records. Rhythm characteristics were identical as those pre- and immediately post-procedure and 14 days after the procedure. After 12 weeks there was no significant change in NYHA class, resting LVEF or WMS. Serum BNP levels decreased from the mean 532 pg/ml to 359 pg/ml after 12 weeks of the procedure. In March 2014 four (40%) patients are alive (Table 1). They are controlled by local cardiologist. They do not need care in the center where the cells were applied. Six patients died 7, 9, 37, 71, 101 and 119 months after the procedure.

The mean number of mononuclear cells (MNC) obtained from the bone marrow aspirate of the patients differed from the number obtained from the healthy donors (donors of sibling allogeneic hematopoetic stem cells), 160×10^6 (range $40–250 \times 10^6$) versus 226×10^6 (range $64–595 \times 10^6$). The growth of patients' MSC (90–100% confluence) was significantly slower i.e. 22.7 versus 13.5 days (Table 2). It is necessary to say that even approximately the same start number of MNC (*patient* 2 and 3 versus *donor* 8 or *patient* 6 and 7 versus *donor* 7 and 9) resulted in the above mentioned “slowness” of cell growth (22 and 27 versus 14 days or 21 or 22 versus 14 and 14 days). The maximal number of passages for the patients' MSC was 5 in contrast to 12 in healthy donors (data not shown). The measured levels of cytokines (VEGF, SCF, FGF, TGFb2) secreted in cell medium during the first incubation/passage of MSC did not differ between patients and healthy donors.

We have performed RNA microarray analysis comparing the MSC from patient No. 10 and donor No. 5 (MNC 70×10^6, growth time 25 days, MSC yield 22×10^6 and MNC 75×10^6, growth time 15 days, MSC yield 22×10^6) (analysis 1) and patient No. 3 and donor No. 8 (MNC 148×10^6, growth time 27 days, MSC yield 24×10^6 and MNC 116×10^6, growth time 15 days, MSC yield 35×10^6) (analysis 2) as shown in Table 2. Mean signal and mean local background intensities were obtained for each spot of the microarray images using the ImaGene® software (Biodiscovery Inc., El Segundo, USA). Low quality spots were flagged and excluded from the data analysis. Unflagged spots were analyzed using the PIQORTM Analyzer software (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The PIQORTM Analyzer allows automated data processing of the raw data text files derived from the ImaGene® software.

Both analyses showed an enhanced ratio of gene expression for STAT1 (SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 1-ALPHA/BETA): 3.65 (analysis 1) and 3.66 (analysis 2). The enhanced ratio of gene expression has been observed for ISG15 (ISG15 OR G1P2 OR UCRP (UBIQUITIN CROSS-REACTIVE PROTEIN)): 4.04 (analysis 1) and 2.77 (analysis 2). The decreased ratio of gene expression has been shown for GTP-binding protein RAD: 0.15 (analysis 1) and 0.69 (analysis 2).

The family of STAT proteins contains seven proteins expressed in the organism. Cardiac physiology condition and ischemic heart disease evidently influence two proteins. The STAT3 is heart protective and STAT1 which increases apoptosis and reduces cardio protective autophagy [7]. The expression of STAT1 protein plays an important role in activation of IL-6 pleiotropic cytokine in the heart. Heart failure and cardiac dysfunction are associated with high circulating IL-6

Table 2
The characteristics of MSC growth. A) patients, B) healthy donors.

Patient No	MNC ($\times 10^{-6}$)	Growth time (d)	MSC yield ($\times 10^{-6}$)
A)			
1	85	19	23
2	150	22	20
3	148	27	24
4	84	17	22
5	180	20	22
6	250	21	40
7	240	22	15
8	153	26	34
9	40	28	15
10	70	25	22
Mean	140	22.7	23.7
Donor No	MNC ($\times 10^{-6}$)	Growth time (d)	MSC yield ($\times 10^{-6}$)
B)			
1	94	14	19
2	595	12	20
3	395	13	12
4	360	13	24
5	75	15	22
6	64	14	40
7	204	14	20
8	116	14	35
9	264	14	20
10	90	12	21
Mean	226	13.5	23.3

levels. Linkage of STAT1, proapoptotic p53 with IL-6 proteins regulates molecular mechanisms involved in pathogenesis of heart failure. Moreover, when there is a STAT1 deficiency, the myocytes fail to induce IL-6 expression upon IFNg treatment [8].

The protein ISG15 is involved in the signal pathway IKK/NF-κB and causes inflammatory response and myocyte atrophy. IKK2 activation led to inflammatory dilated cardiomyopathy and heart failure in adult animals [9]. The increased expression of ISG15 may activate proinflammatory IKK/NF-κB pathway. In concert with increased expression of STAT1 which enhances expression of another proinflammatory cytokine IL-6, it can potentiate inflammatory process in the heart tissue.

The GTP-binding protein RAD may play an important role in cardiac anti-arrhythmia via the strong suppression of voltage-gated L-type Ca^{2+} currents, regulates voltage-dependent L-type calcium channel subunit alpha-1C trafficking to the cell membrane, inhibits cardiac hypertrophy through the calmodulin-dependent kinase II (CaMKII) pathway and inhibits phosphorylation and activation of CAMK2D [6]. Suppression of the RAD activity suggests that RAD-associated signaling pathway may play role in arrhythmogenesis. Moreover, decreased expression of RAD in mice model can induce ventricular tachycardia [10].

The first analyzed patient died 101 months after the procedure. The second one is alive 120+ months after the procedure. It is tempting to speculate about the following fact: How is it possible that the patients with this "damaged" RNA profile survived such a long time after the procedure? One possible explanation could be that the myocardial repair occurred because of the presence of huge amount of ex vivo expanded MSC which reflect only the statistically up or down regulation of certain genes.

This work has been supported by the Foundation for Transplantation. We are grateful Dr. M. Cihova for English correction.

The author(s) of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

All authors declare no conflict of interest.

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http://dx.doi.org/10.1016/j.ijcard.2014.07.024