Personal contributions in the study of stem cell therapy in atherothrombotic diseases

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Abstract

Introduction: Stem cell injection at the level of ischaemic tissues is a promising new therapeutic approach in patients with atherothrombotic diseases, several international trials (TOPCARE, BOOST, MySTAR, REAPIR-AMI) showing encouraging results such as imprivement of ejection fraction and clinical symptomatology on long term in postmyocardial infarction patients. Also, in critical limb ischaemia, intraarterial injection of stem cells into the affected arteries seems to represent a promising therapy leading to limb salvage and wound healing. This research aims to study the effect of stem cell injection in ischaemic tissues in patients (1) post acute myocardial infarction, targeting the recovery of a larger myocardial tissue and improvement of left ventricular function (2) with critical limb ischaemia, in whom muscular biopsies have been performed in order to reveal neoangiogenesis process at the level of injected tissues or arteries.

Material and methods: The study was conducetd in the period of 1st november 2005 – 1st of may 2012, in the Clinic of Cardiology of the County Clinical Emergency Hospital Mures. The effect of stem cell therapy in atherothrombotic diseases has been followed in two patients groups, in two distinct studies:

- Study 1- Stem cell therapy in post acute myocardial infarction (AMI) patients

Study design – prospective study including post AMI patients between 3 weeks and 3 months after myocardial infarction, randomized in two groups: group 1 -9 patients with intracoronary injection of autologus mononuclear stem cells from bone marrow, and group 2 – 28 patients – control lot (without stem cell injection).

Methodology: In all patients 3D echocardiography has been performed at baseline, 3 months, 6 months, 1 year and 4 years after stem cell injection, and in group 1 Angio CT multislice 64 has been performed at 4 years after stem cell injection.

-Study 2- stem cell therapy in critical limb ichaemia

Study design: prospective study including patients with critical limb ischaemia, randomized in two groups: (1) treatment group – intraarterial and intramuscular injection of autologus stem cells from bone marrow, and (2) control group.

***Methodology*:** In all patients we performed 6 weeks, 6 months and 1 year follow-up, to reveal the improvement in clinical status and symptoms.

Statistical analysis has been performed using JMP 10 (SAS Institute Inc., Cary, North Carolina) and Graph InStat Pad softwares. Mann-Whitney test was used for determination of statistical significance. Categorical variables are expressed as percentages and were compared using Fisher's exact test. Multivariate analysis has been used to asses predictors for response to stem cell therapy and statistical significance was reached for p values below 0.05.

Results

Study 1 – There were no statistical significant differences between the study groups at baseline regarding age (53,56 +/-15.08 vs 59,75+/- 8.4 years, p=0.2), gender (77,77% male gender vs 67,86% p=0,69), angina class (55,55% class 2 vs 53,57%, p=1), NYHA status (55,55% NYHA 3 vs 60,71% p=1, ns), maximum level of cardiac enyzmes (3.106 +/- 1.488 vs 3.392 +/- 998.54, p=0.6), presence of risk factors: diabetes (22,22% vs 21,42% p=1, ns), hypertension (33,33% vs 32,14% p=1), dyslipidemia (88,89% vs 85,72% p=1), smoking

status *(*33,33% vs 35,71% p=1), familial history *(*88,88% vs 78,57% p=0.65*)*. Also, there were no statistical significant differences between the study groups at baseline regarding the indexes of global and regional contractility: wall motion score index (1,73+/-0,26 vs 1,70+/-0,2 p=0,7), 3D segmental contractility index 3D (1,94+/-0.27 vs 1,98+/-0.2, p=0.7), 3D dis-syncronism index (71,4 +/- 5.2 vs 76,6+/-10.6 p=0.05), enddiastolic volume (192 +/-27.2 ml vs 194,5+/-18.5 ml, p=0.8), endsystolic volume (112,2 +/- 21.1 ml vs 115,6 +/-13.1 ml, p=0.6), or EF (41,66% +/- 3.5% vs 40,57%+/- 3.1% p=0.4).

Primary endpoint

Follow-up showed an improvement of EF with 13% vs 7,9% (p=0,007) at 3 months and with 14,2% vs 8,6% (p=0,007) at 6 months. Wall motion score index showed also an improvement with 15% vs 5% at 3 months and with 20% vs 7% at 6 months (p=0,01).

3D segmental contractility index increased with 23% vs 11% (p=0,006) at 3 months, respectively with 42% vs 11% at 6 months (p<0.0001). 3D dis-synchronism index showed an improvement with 19% vs 10,7% (p=0,4) at 3 months respectively with 51% vs 13,7% at 6 months (p<0.001).

Secondary endpoints

MACE rate at 4 years was significantly lower in the study group compared with control group, as related to death rates (11,11% vs 14,2%), need for revascularisation (11.11% vs 14,2%) or composit endpoint (death, reinfarction, revascularization) (22,22% vs 39,3%).

Regression of cardiac remodelling postinfarction was superior in the study group, as expressed by reduction of left ventricular end-diastolic (4.1% vs 2,1% reduction, p<0.001) and end-systolic volumes (14.3% vs 8% reduction, p<0.001).

Clinical symptomatology showed superior results in the study group as compared with the control group, expressed by improvement in angina class and NYHA status.

Progression of atherosclerotic process in the coronary artery injected with stem cells was assesed by angio CT multislice performed at 4 years after cell therapy, showing a marked reduction in treated arteries, in which we identified a significantly lower number of coronary plaques (1,62 vs 4,68, p<0.001) and a significantly lower calcium score (36.87 vs 186.8, p<0.001) compared with the other arteries of the same patient.

Study 2

There were no statistical significant differences between the study groups at baseline.

Primary endpoint – we recorded a significantly superior improvement of functional status in the treatment arm (increase in claudication index with 225% in group 1 vs 80% in group 2 – p=0.006) and improvement of clinical symptomatology.

Secondary endpoints: *MACE and amputation rates* was lower in the study group as compared with the control group, with a composit endpoint (death, amputation, revascularization) of 20% vs 44,44% at 1 year. *Development of collateral network after neoangiogenesis post stem cell injection* has been proved by angiographic analysis at 6 months after stem cell injection, showing a significant increase in the number and density of collateral vessels in the affected limb, while imunohistochemic analysis of bioptic tissue demonstrated the presence of *markers of neoangiogenesis* (vascular grow factors) at this level.

Discussions

In post AMI patients we recorded a significant clinical benefit following stem cell therapy, concordant with the results of international clinical trials. The most frequent endpoint used in clinical studies on cell therapy, Ejection Fraction, showed a significant improvement in the stem cell group as compared with the control group.

As a priority in the literature, this study identifies objective and quantifiable parameters for assesment of ventricular function post cell therapy, such as those obtained using 3D echocardiography. The differences between the groups were more expressed as regarding parameters characterising regional function than those characterising global

function, showing a more pronounced improvement of segmental contraction in patients post stem cell therapy. Long term follow-up at 4 years showed a clear superiority of stem cell therapy in reduction of cardiac events, leading to a reduction of composit endpoint (death, reinfarction or revascularization) from 39,3% to 22,22% at 4 years.

Another priority of thos study on international level is the long term follow up of the effect of stem cells on reduction of progression of atherosclerotic process in the injected coronary arteries, assessed by angio CT multislice 64, which revealed significant reduction in the number of coronary plaques and the level of calcium score in the treated arteries.

Regarding the role of stem cell therapy in critical limb ischaemia, similar with the international trials like PROVASA which showed complete healing of ischaemic ulcer at 10.9 months after stem cell injection, this study shows a significant improvement of clinical status after intraarterial and intramuscular injection of stem cells in critical limb ischaemia, imunohistochemical analysis showing the presence of markers of neoangiogenesis at the level of injected arteries and tissues.

Conclusions

This study demonstrates the safety and efficacity of stem cell therapy in two major subsets of atherothrombotic diseases: acute myocardial infarction and critical limb ischaemia.

In patients post myocardial infarction stem cell therapy is associated with an improvement of global and especially regional ventricular function. Long term follow up at 4 years indicates a clear superiority of stem cell therapy in reduction of MACE rates and reduction of progression of atherosclerotic procces at the level of treated coronary arteries.

In patients with critical limb ischaemia, stem cell therapy is associated with a clear improvement of clinical symptoms and functional status, our data showing stimulation of neoangiogenesis and development of collateral vessel at the level of ischaemic tissues injected with stem cells.

Therefore, stem cell therapy could be considered a viable alternative for treatment of ischaemic tissues in atherothrombotic diseases, playing a major role in recovery of their function and improvement of clinical evolution in these patients.