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THE RELATIONSHIP BETWEEN LEFT VENTRICULAR FRACTIONAL SHORTENING AND INTRAVENOUS ADMINISTRATION OF STEM CELLS IN LABORATORY RABBITS PRESENTING CHRONIC MYOCARDIAL INFARCTION

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Abstract

Background and aims*. The present study conducted from March 2012 to July 2013 aimed to evaluate from echocardiographic point of view the effects of peripheral intravenous administration of mesenchymal stem cells (MSCs) in laboratory rabbits presenting 30 days old chronic myocardial infarction.*

Material and methods. *30 days after the induction of an acute myocardial infarction in 40 laboratory rabbits by direct ligation of the left anterior descending coronary artery at about 10 mm from the apex, we injected 1x106 MSCs in the auricular vein in a group of 30 rabbits, and a group of 10 rabbits were used as controls. 30 days after the injection of stem cells the left ventricular (LV) fractional shortening (FS) was evaluated by echocardiography and compared with the control rabbits.*

Results. *In control rabbits, echocardiography revealed akinesis of apex, interventricular septum kinetics was also impaired, FS being approximately 6%. In 80% (24 rabbits) of the injected rabbits the FS of the LV was significantly greater than in the witness group (26+/-2%, p<0.0001). At 13.3% (4 rabbits) of the injected rabbits the FS of the LV showed no improvement in comparison with the control group (6.5+/-1%).*

Conclusion. *An improvement of LV SF 30 days after MSCs were injected(p<0.0001) was noted. We have to further determine if this improvement of the LV function is correlated with any histopathological changes and if it is not lost in time. Also, further studies needs to evaluate if there is any significant change in the overall mortality.*

Keywords: mesenchymal stem cells, acute myocardial infarction, chronic myocardial infarction, fractional shortening, left ventricle.

Background and aims

Ischemic heart disease remain a major public health problem in western countries despite a better understanding of its pathophysiology and therapeutic managment. Cardiovascular disease is the number one cause of death worldwide [1,2]. Congestive heart failure (CHF) is the end stage of many heart diseases, but ischemic heart disease is one of the most common etiology. CHF is five times more common in those who have had an acute myocardial infarction (AMI) than in those who have not. The prognosis for those with established CHF is generally poor and worse than for those with most malignancies or AIDS, with a one-year mortality rate as high as 40 percent and a five-year mortality between 26 and 75 percent [3]. If, in case of an AMI, the medical and interventional management are the „the gold standard", once the chronic myocardial infarction develops, the medical management of the patient is limited

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and progression towards CHF is often certain. Cellular cardiomyoplasty may be a promising approach to improve cardiac regeneration or vascularization after chronic myocardial infarction develops [4]. In the international literature it has been reported that transplantation of fetal or neonatal cardiomyocytes can increase the thickness of the infarct wall and LV stroke volume, decrease LV end-systolic volume, and improve LV ejection fraction in a rat model of myocardial infarction [5,6] and induce neoangiogenesis [7].

As sometimes only the peripheral intravenous route is available, the present study, conducted between March 2012-July 2013 at the Iuliu Hatieganu University of Medicine and Pharmacy Cluj Napoca's Biobase in collaboration with the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, aimed at evaluating the effects of peripheral intravenous administration of stem cells 30 days after the induction of an acute myocardial infarction in laboratory rabbit. This article highlights the echocardiographic changes of LV FS at 30 days after peripheral intravenous administration of stem cells in rabbits with chronic myocardial infarction.

Materials and methods

All procedures of the experiments complied with the standards of animal care. Working protocols were approved by the Ethics Committee of the Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca [8].

Bone marrow harvest

In order to harvest the MSCs we used 20 New Zealand White rabbits (3-3.5 kg) 1 year of age. Rabbits were from the Biobase of the Iuliu Hatieganu University of Medicine and Pharmacy , Cluj-Napoca. Marrow punctures were made after general and local anesthesia. Neuroleptanalgesia was performed using acepromazine and ketamine, and local anesthesia was performed with alphacain and adrenaline in separate points. Local antisepsis and asepsis were performed prior to bone puncture. For intervention the animals were restrained in lateral decubitus, at the iliac crest a blade skin incision was performed followed by sterile needle penetration in the spinal canal approximately 1 cm from the iliac crests. MSC acquired from bone marrow aspiration were grown on standard culture mediums [9,10,11,12,13].

Model of myocardial infarction and injection of MSCs

For this study we used 40 rabbits, breed New Zealand White (3-3.5 kg), age of 1 year. The acute myocardial infarction was induced by direct ligation of the left anterior descending (LAD) coronary artery at about 10 mm from the apex, followed by a period of 30 days in witch the rabbits were kept at standard conditions of temperature, humidity and food for the myocardial infarction to become chronic [14].

After the chronic myocardial infarction was achieved, 30 rabbits were injected with $1x10^6$ stem cells/ rabbit using the auricular vein under mild sedation of Ketamine (15 mg/kg) [15,16,17,18]. Another group of 10 rabbits with chronic myocardial infarction was kept as controls.

Echocardiographic assessment

30 days after the administration of the stem cells both groups were subjected to a blind echocardiographic evaluation of the LV, with special interest in FS.

In the literature the left ventricle FS is considered as: normal from 25-40%; mildly impaired from 20-25%, moderately impaired from 15-20%; and severely impaired if <15% [19].

In our previous study, when we developed the experimental model, the value of FS in normal rabbits was 50+/-2%, value that we considered as normal for this study [14].

The ultrasound examination was performed through a subxiphoid window with rabbit shaved, sedated with Ketamine (15 mg/kg) and prone. We tried to focus on the LV, and mostly on its FS (end diastolic diameter - end systolic diameter/end diastolic diameter X 100) in MMode [20] .

Statistical analysis

All data are presented as mean +/- SEM. Comparisons between groups were made by Student *t* test or Fisher's exact test, where appropriate. Results were considered statistically significant if $p<0.05$.

Results

In control rabbits, echocardiography performed 60 days after the induction of the acute myocardial infarction evidenced a severe impaired LV function, the FS being approx. 6%, akinesia of LV apex was also noted and interventricular septum kinetics was also impaired (Fig. 1).

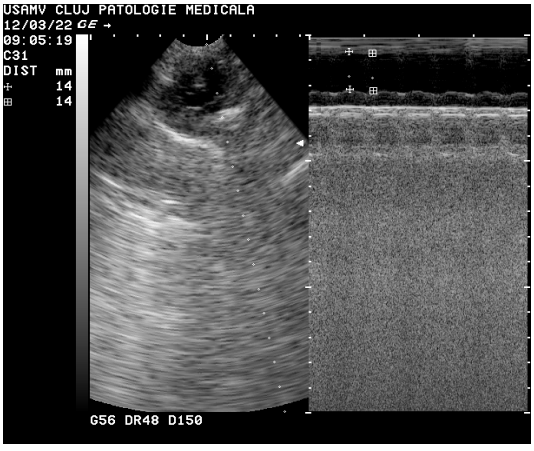

Figure 1. Echocardiography in a control rabbit showing akinesia of the apex.

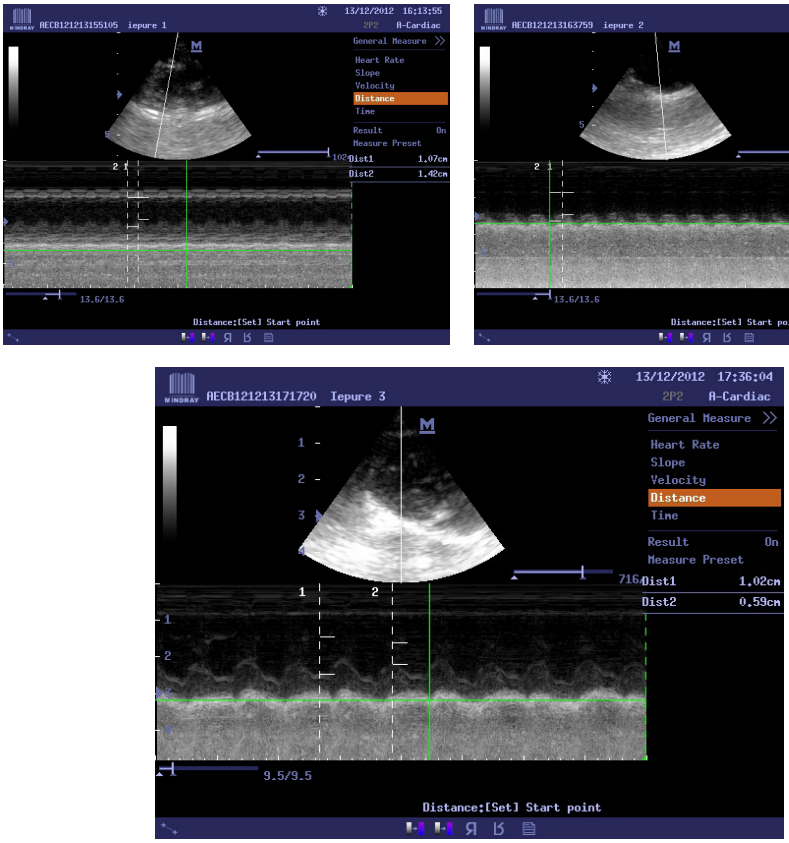

Figure 2. LV echocardiography in injected rabbits showing an average FS of 32%.

Two of the injected rabbits were excluded from the study because the FS was suspiciously high (35%, respectively 36%).

In 80% (24 rabbits) of the injected rabbits the FS of the LV was significantly greater than in the control group (26+/-2%, P<0.0001).

In 13.3% (4 rabbits) of the injected rabbits the FS of the LV showed no improvement in comparison with the control group (6.5+/-1%) (Fig. 2).

At this stage of the experiment we had no mortality among the rabbits, but before the experimental model was well defined the mortality reached in some lots up to 30% mostly intraoperatively or at a second exposure to anaesthetic drugs.

Discussion

Since ischemic heart disease remains a major public health problem in western countries, a continuous research for alternative therapies remains a priority. The stem cell ability to differentiate into mesoderm and non mesoderm-derived tissues has aroused the interest of many researchers to evaluate their effect in cardiovascular disease management, and has proved to be an effective therapy [11,12,21].

Since most studies uses an intralesional administration of MSCs with good results, but in practice not always possible, we wanted to evaluate the impact of peripheral intravenous administration [22,23].

The FS reflects the left ventricle systolic function. If we consider the left ventricle FS as: normal from 50+/-2%; and severely impaired if <15%, the results obtained are promising [14].

We admit that an echocardiography study done before and after administrating the MSCs in every rabbit would have had reduced to minimum any subjective findings in LV evaluation, but the low tolerance of general anaesthetic with high mortality rate (up to 30%), made us abandon this strategy.

We excluded from the study the 2 rabbits with FS of 35%, respectively 36%, because we believe the distance of LAD ligation was probably closer to the apex that it was supposed to be (it corresponds to a FS of the rabbits who underwent LAD ligation at approximately 5 mm from apex at experimental model) [14].

Even if LAD artery ligation at 10 mm from apex caused a severe LV impairment (100% of the control rabbits) and in the group receiving intravenous MSCs we noticed an increase of FS in most of the cases, 4 weeks after the MSCs were administered, we have to admit the limitation of the study since we realize that the LV function is best evaluated measuring its ejection fraction (which was not possible to measure due to device we had access to). We were also unable to correlate this improvement with the clinical symptomatology (very hard to evaluate on an

animal model).

There are also questions left to be answered, like: will this improvement we noticed last? Wangde et al [24] in his experiment injected MSCs into the scar of a 1-week-old myocardial infarction in Fischer rats and an improved of global LV function 4 weeks after was obtained, but this benefit was lost 6 months later. Further more he noted that histopathologically, the LV function improvement 4 weeks after MSCs were administered was achieved without an increased thickening of the infarcted muscle wall. They concluded that the injected MSCs may had produced humoral substances that had a beneficial effect over the surrounding viable muscle cells in some way, a so-called paracrine effect. We have no reason to exclude this paracrine effect theory of the injected MSCs over the improvement of LV FS in our study, the histology will hopefully answer this question.

We also don't know at this moment the impact of pulmonary passage over the injected MSCs and if there is a relationship between this and the "unresponsive" rabbits. We haven't found a reason why 13.3% of the injected rabbits showed no improvement of LV FS in comparison with the control group.

Even if our study does no report tumor formation, question remains regarding the long term safety of using stem cells [25,26].

Conclusion

An improvement (comparing with the control group) of LV SF in injected rabbits, 30 days after MSCs were administered ($p<0.0001$) was noted.

We have to further determine if this improvement of the LV function is correlated with any histopathological changes and if it is not lost in time.

Also further studies need to evaluate if there are any significant changes in the overall mortality.

We cannot say at this stage if peripheral intravenous route is or is not a viable one for the administration of MSCs for ischemic heart pathology.

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