Study of the efficiency of cell based therepies in a model of myocardial infarction

Ph.D. Thesis

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1. Introduction

Cardiovascular diseases are a major health concern and social problem. In Europe 46% of all mortality is the result of cardiovascular illness. Data from Hungary show an even sadder picture: every second death is due to cardiovascular disease. Among them one of the most significant is myocardial infarction partly because of its frequency and adverse effects on the long-term quality of life impact, but also due to its high costs and social implications. Although as a result of the development of medications and surgical procedures 90-95% of patients survive the first heart attack after a serious heart attack the loss of contractile tissue and its replacement by fibrotic scar tissue induces various compensatory mechanisms that can lead to decreased heart function and the development of heart failure on the long run. The morbidity and mortality figures of heart failure are rising and there is currently no therapy that could reverse the decline in heart function.

Because after myocardial infarction the cells of the adult heart are unable to replace the damaged tissue replacement, high hopes aroused for cell-based therapies for the treatment of damaged heart. There have been reports on the beneficial effects of bone marrow and adipose tissue-derived mesenchymal stem cells transplantation after acute myocardial infarction both in small and large animal models. The cell transplantation in many cases reduced the infarcted area while increased ejection fraction and angiogenesis. A growing number of clinical studies confirm the safety and feasibility of stem cell therapy after myocardial infarction. A number of multi-center studies are underway to examine the efficacy of the treatments. Bone marrow derived mesenchymal stem cells are the most extensively studied and best known stem cell types, the majority of clinical trials use this cell type. In addition to their differentiation potential toward different cell lines these cells are capable of modulation of the immunological processes in the damaged heart. Although bone marrow

represents a stable and reliable source for mesenchymal stem cells bone marrow extraction is an invasive procedure and the yield of isolated stem cells is low and age dependent. In contrast, adipose tissue is easily available in large quantities and large amounts of stem cells can be recovered this way. The isolated cells can be stably maintained for a long term in an in vitro environment. Bone marrow and adipose tissue-derived mesenchymal stem cells share many properties, but several differences have been described in regard of immunophenotype, differentiation potential, transcriptome, proteome and immunomodulatory activity. However, limited data is available about their efficacy relative to each other in cell therapy settings.

Currently, one of the biggest barriers of effective stem cell therapy of myocardial infarction is the low engraftment and survival of the injected cells, which reduces the potential beneficial effects on regeneration and function. Preconditioning the therapeutic cells using physical, chemical, pharmacological or genetic manipulation shows promising results increasing cell survival in in vitro ischemic models and in vivo transplantations. In our experiments we used the poly(ADP-ribose) polymerase (PARP) inhibitor PJ34 for pretreating the therapeutic cells. PARP inhibition allows the cells undergoing oxidative stress to conserve ATP and NAD stores, thereby enabling them to regain normal function or perish using the apoptotic cell death pathway, which is much better for the organism than the pro-inflammatory necrotic cell death. Accordingly, PARP inhibitor pretreatment is a promising option for increasing the survival of the transplanted cells in the hostile post-ischemic microenvironment after myocardial infarction.

2. Aims

Due to conditions outlined in the introduction it is necessary and useful to compare the cell types frequently used and gather more data about pretreatments. In our experiments we used an in vitro approach for modeling the complex events of cell therapy after myocardial infarction.

During my work my goals were:

- to characterize the in vitro model of ischemia-reperfusion used by our group,
- to compare the bone marrow and adipose tissue-derived mesenchymal stem cells' survival in a post-ischemic environment,
- to compare the direct effects of bone marrow and adipose tissue-derived mesenchymal stem cells on cardiomyoblasts injured with ischemia-reperfusion insult,
- to assess the effect of PARP inhibitor pretreatment of therapeutically used H9c2 cells on their survival in a post-ischemic environment,
- to examine the effect of pretreatment of the therapeutically used H9c2 cells by PARP inhibition on the viability of cardiomyoblasts injured with ischemia-reperfusion insult.

3. Materials and methods

3.1. Ischemia-reperfusion model

We modeled ischemic environment on in vitro cultured H9c2 cardiomyoblasts using oxygen and glucose deprivation. During the incubation glucose and L-glutamine free DMEM media and an atmosphere of 0.5% oxygen (approx. 4 mmHg) and 99.5% nitrogen, at 37°C temperature were used. The incubation system controls the temperature and the O_2 concentration, and makes it possible to use a microscope to follow the morphological changes of the cells.

The duration of the ischemic treatment was 160 minutes. 12-well plates were used and the H9c2 cardiomyoblasts were plated 1 ml one day prior to the ischemic insult. Starting the ischemic treatment the cells were washed twice with PBS and then 3 ml of a glucose-free medium was pipetted into each well. After the hypoxic incubation the medium was changed to 3 ml normal cell culture DMEM medium containing 5 g/l glucose, 10% fetal bovine serum, 4 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin, then the plates were incubated at 37°C in a fully humidified atmosphere of 5% CO_2 and 95% air.

3.2. The fluorescent labeling of the therapeutic cells - Vybrant DiD

Since it was very important in our investigation to distinguish the injured and the therapeutically added cells we used Vybrant DiD (excitation/emission: 644 nm / 663 nm) fluorescent membrane stain for labeling the therapeutically used cells. The dye was applied in a 1: 2000 dilution (500 nM).

3.3. PARP inhibitor pretreatment of the therapeutic cells

The cells were exposed to PJ34 (Inotek Pharmaceuticals Corp., Beverly, MA) PARP inhibitor for 1 hour under normal cell culture conditions, the cell culture

medium was supplemented with the appropriate concentration of PJ34 (10 μM or 100 μM) or in the case of control with PBS. After the incubation time, the PARP inhibitor was removed by washing the cells twice with PBS. The treatment was performed immediately before using the therapeutic cell for the treatment of the cardiomyoblasts injured with ischemia-reperfusion.

3.4. Human adipose tissue derived stem cell conditioned medium

Preparing the human adipose tissue-derived mesenchymal stem cell (hASC) conditioned media (ACM). hASC cells were incubated in 100 mm Petri dishes at 10 000 cells/cm^2 confluency, in 8 ml of DMEM medium containing 1 g/l glucose, 10% fetal bovine serum, 4 mM L-glutamine and 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C temperature in a fully humidified atmosphere of 5% CO_2 and 95% air. The medium was aspirated after 48 hours, and was retained for further use.

3.5. Live/dead viability staining – calcein-ethidium homodimer

For the purpose of distinguishing live and dead cells, calcein and ethidium homodimer stains were used.

The non-fluorescent calcein-AM can pass through the cell membrane and in the cytoplasm it is converted fluorescent membrane-impermeable calcein by intracellular esterase activity. Thus the calcein shows an intensive green fluorescence (excitation / emission: 494 nm / 517 nm) in the cytoplasm of living cells. If the membrane integrity is disrupted, the retention of the stain is reduced, and the fluorescent cell staining gradually disappears.

The ethidium homodimer (excitation / emission: 528 nm / 617 nm) has a strong positive charge, it can not cross the intact cell membranes so it do not stain the healthy, living cells, it can only pass through the disrupted membrane of damaged cells. Binding to DNA its fluorescent activity increases 40-fold, thus the injured and the dead cells nucleus gives of vivid red fluorescent signal.

3.6. Flow cytometry

24 hours after the ischemic insult the cells were separated from the culture plate surface by trypsination and after 8 minutes of centrifugation at 1200 rpm the pellet was resuspended in a PBS solution of the fluorescent live/dead stains. The calcein was used in a 1:12 000 dilution and the ethidium homodimer was used in a 1:3000 solution. The measurements were taken using a BD Biosciences FACSCalibur flow cytometer and the CellQuest program.

DiD labeling of the therapeutic cells were clearly distinguishable from the unlabeled cardiomyoblast cells. The data gathered from the cardiomyoblasts which went through the ischemia-reperfusion were separated from the data of the therapeutic cells gating for the DiD signal.

Based on the viability staining three different cell populations could be distinguished: the living cells showing strong calcein signal, the necrotic cells showing strong ethidium-homodimer signal, and a third group of cells showing intermediate staining properties which can be considered an apoptotic cell population based on the literature (figure 1.).

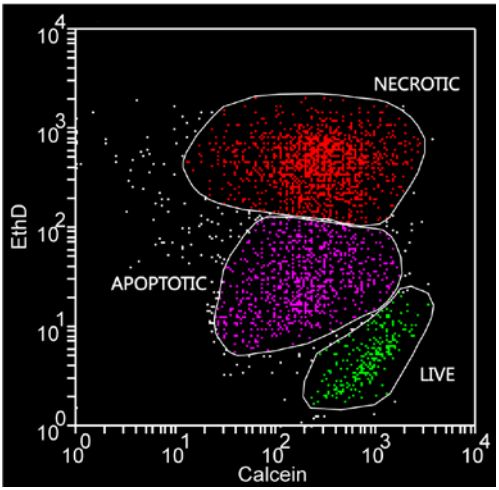

Figure 1. Calcein-ethidium homodimer dot-plot. EthD – ethidium homodimer

3.7. Metabolic activity measurement

Cell number and metabolic activity can be measured quantitatively using the reducing capability of the living cells. Several methods are known in the literature, which operates on this principle, in our present experiments we used PrestoBlue reagent. The reagent contains the membrane-permeable blue resazurin component which is non-fluorescent. In the living cells this chemical is reduced to the red, highly fluorescent resorufin compound. After the reduction the maximum absorbance of the dye is shifted from 600 nm (resazurin) 570 nm (resorufin), the analysis can be performed by measuring absorbance in these two wavelengths.

3.8. Malondialdehyde measurement

The reactive oxygen species generated during the ischemia-reperfusion injury cause lipedperoxidation. Malondialdehyde (MDA) is the most commonly used marker for the peroxidation of polyunsaturated fatty acids. In neutral pH it is present in its enolate anion form which has low chemical reactivity. Quantitative analysis of MDA is based on its reaction with thiobarbituric acid, which is possible in acidic conditions and high temperature. The product of the condensation reaction between two molecules MDA and one molecule thiobarbituric acid can be spectrophotometrically detected at 532 nm.

3.9. Lactate dehydrogenase release measurement

Lactate dehydrogenase activity was measured using a coupled reaction during which the enzyme lactate dehydrogenase (LDH) catalyses the conversion of lactate to pyruvate and produces a reduced coenzyme which is further used reducing indonitrotetrazolium into formazan which can be detected at 490 nm.

3.10. Experimental protocols

3.10.1. Characterization of the ischemia-reperfusion model

In the experiments 12 well plates were used and 100 000 H9c2 cardiomyoblasts in 1 ml medium were plated in each well one day prior to the ischemic insult. Starting the ischemic injury the cells were washed twice with PBS and then 3 ml glucose-free medium was pipetted into each well. The cells were incubated in an

atmosphere of 0.5% oxygen (approx. 4 mmHg) and 99.5% nitrogen at 37°C for 160 minutes. After the hypoxic incubation the medium was changed to 3 ml normal cell culture DMEM medium containing 5 g/l glucose, 10% fetal bovine serum, 4 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin, then the plates were incubated at 37°C temperature in a fully humidified atmosphere of 5% CO_2 and 95% air.

Flow cytometric measurement and lactate dehydrogenase release measurement from the cell culture medium were performed 24 hours after the completion of simulated ischemia. Lactate dehydrogenase release was measured from the cell culture media.

Malondialdehyde measurement was taken 5 hours after the completion of simulated ischemia from the cell culture medium.

3.10.2. Comparison of the effects of bone marrow and adipose tissue-derived stem cell treatments in the in vitro ischemia-reperfusion model

In the experiments 12 well plates were used and 30 000 H9c2 cardiomyoblasts in 1 ml medium were plated in each well one day prior to the ischemic insult. Starting the ischemic injury the cells were washed twice with PBS and then 3 ml glucose-free medium was pipetted into each well. The cells were incubated in an atmosphere of 0.5% oxygen (approx. 4 mmHg) and 99.5% nitrogen at 37°C for 160 minutes. After the hypoxic incubation the different groups were treated as follows:

- I-R model: untreated group
- ACM: hASC conditioned medium treated group,
- hBMSC: hBMSC treated group,
- hASC: hASC treated group.

After that the cells were incubated at 37°C temperature in a fully humidified atmosphere of 5% CO_2 and 95% air.

The evaluations were performed 24 hours after the completion of the simulated ischemia-reperfusion (flow cytometry, lactate dehydrogenase release measurement and metabolic activity measurement).

3.10.3. Effects of PJ34 PARP inhibitor pretreatment of therapeutic cells in an in vitro model of ischemia-reperfusion

3.10.3.1. Preliminary experiments

For the assessment of the cytotoxicity of PJ34 96-well plates were used containing 10 000 H9c2 cardiomyoblast cells/well. Normal cell medium was supplemented with appropriate concentrations (10 μM or 100 μM) of PJ 34 or in the case of control with PBS. After one hour of incubation in standard cell culture conditions cytotoxicity measurement was performed using LDH release assay.

The experiments to confirm the protective effect PARP inhibition against oxidative stress were carried out using 96-well plates containing 10 000 H9c2 cardiomyoblast cells/well. Normal cell medium was supplemented with appropriate concentrations (10 μM or 100 μM) of PJ 34 or with PBS in the case of the control group. After one hour of incubation in standard cell culture conditions hydrogen peroxide were added to the wells (400 μM final concentration). After two hours of further incubation in standard cell culture conditions cytotoxicity measurement was performed using LDH release assay.

3.10.3.2. Main experiments

In the experiments 12 well plates were used and 30 000 H9c2 cardiomyoblasts in 1 ml medium were plated in each well one day prior to the ischemic insult. Starting the ischemic injury the cells were washed twice with PBS and then 3 ml glucose-free medium was pipetted into each well. The cells were incubated in an atmosphere of 0.5% oxygen (approx. 4 mmHg) and 99.5% nitrogen at 37°C for 160 minutes.

After the hypoxic incubation the different groups were treated as follows:

I-R model: untreated group,

H9c2: H9c2 cardiomyoblast treated group,

10 μM PJ34: 10 μM PJ34 pretreated H9c2 cardiomyoblast treated group,

100 μM PJ34: 100 μM PJ34 pretreated H9c2 cardiomyoblast treated group.

After that the cells were incubated at 37°C temperature in a fully humidified atmosphere of 5% CO_2 and 95% air.

The evaluations were performed 24 hours after the simulated ischemia-reperfusion using flow cytometric measurement.

3.12. Data analysis and statistics

For the evaluation of the flow cytometric data the Weasel program was used (The Walter and Eliza Hall Institute, Parkville, VIC, Australia). Statistical analysis and diagram editing was performed using the Graphpad Prism program (GraphPad Software, San Diego, CA, USA). During the statistical analysis Student's two-sided unpaired t-test or one-way analysis of variance (ANOVA) and Newman-Keuls post hoc test were used as appropriate. In all cases the results are given as mean ± SEM. A p value of less than 0.05 was considered statistically significant.

4. Results

4.1. Characterization of the ischemia-reperfusion model

The results of the flow cytometric measurements show that the ischemia-reperfusion model significantly ($p<0.001$) reduced the number of viable cells ($12.13 \pm 0.75\%$) relative to the untreated control group ($90.36 \pm 2.60\%$).

The ischemia-reperfusion model significantly increased the oxidative stress, as shown by the MDA levels being significantly ($p<0.001$) higher (13.70 ± 0.81 μM) compared to the untreated control group (0.47 ± 0.18 μM).

Subjecting the cell to the ischemia-reperfusion model also increased significantly ($p<0.001$) the LDH release measured from the cell culture medium ($29.58 \pm 6.21\%$) relative to the untreated control group ($0.00 \pm 0.81\%$).

4.2. Comparison of the effects of bone marrow and adipose tissue-derived stem cell treatments in the in vitro ischemia-reperfusion model

The survival of the cardiomyoblast cells undergoing the ischemia-reperfusion insult was $12.13 \pm 0.75\%$ in the untreated group. The survival rate of the cardiomyoblasts significantly increased in both the hASC treated ($24.66 \pm 2.49\%$) and the hBMSC treated groups ($25.41 \pm 1.99\%$), while in the conditioned medium treated group ($13.94 \pm 1.44\%$) there was no significant change. There was no significant difference between the groups treated with bone marrow derived and adipose tissue derived mesenchymal stem cells, however, in both stem cell-treated group there were a significantly higher post-ischemic cell survival than the group treated with the conditioned medium (Figure 2A). The apoptotic cell ratio of cardiomyoblasts that undergone the ischemia-reperfusion insult was $39.62 \pm 2.44\%$ in the untreated group, which value was significantly lower in the conditioned medium treated ($23.36 \pm 2.51\%$) and hASC-treated ($47.08 \pm 2.61\%$) groups, but did not change significantly in the hBMSC treated group ($32.75 \pm 2.92\%$). The hASC treated

group had a significantly higher proportion of apoptotic cardiomyoblast cells relative to the hBMSC treated group (Figure 2A).

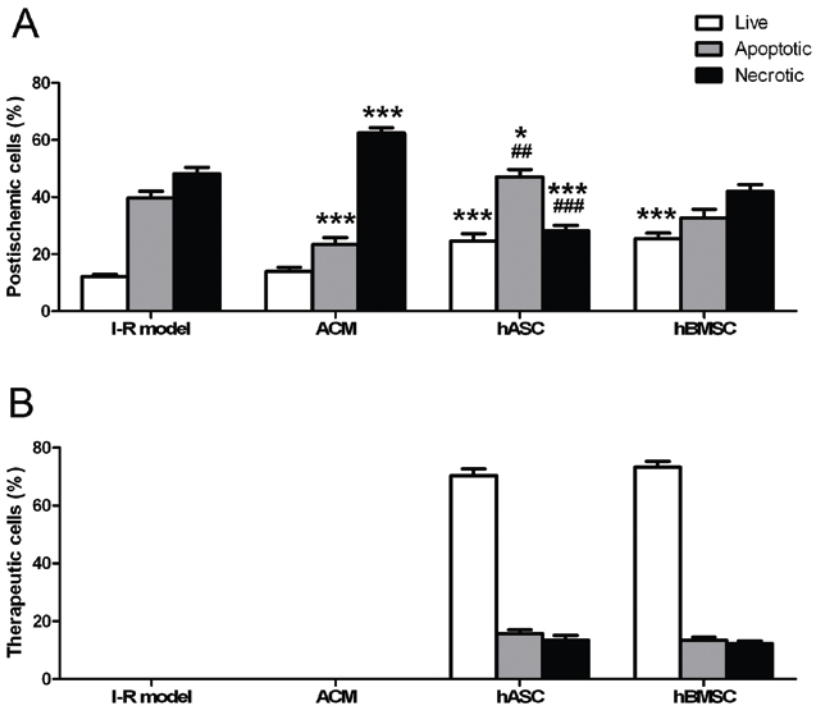

Figure 2. Flow cytometric analysis. A) The ratio of live, apoptotic and necrotic postischemic cells. I-R model: live: 12.13 ± 0.75%, apoptotic: 39.62 ± 2.44%, necrotic: 48.09 ± 2.28%; ACM: live: 13.94 ± 1.44%, apoptotic: 23.36 ± 2.51%; necrotic: 62.39 ± 1.89%; hASC: live: 24.66 ± 2.49%, apoptotic: 47.08 ± 2.61%, necrotic: 28.16 ± 1.94%; hBMSC: live: 25.41 ± 1.99%, apoptotic: 32.75 ± 2.92%, necrotic: 41.88 ± 2.46%. **: p <0.01 vs. I-R model, ***: p <0.001 vs. I-R model, ## : p <0.01 vs. hBMSC, ### : p <0.001 vs. hBMSC, ANOVA, Newman-Keuls post hoc test, n = 17-31 B) The ratio of live, apoptotic and necrotic therapeutic stem cells. hASC: live: 70.30 ± 2.35%, apoptotic: 15.79 ± 1.22%, necrotic: 13.41 ± 1.67%; hBMSC: live: 73.30 ± 1.92%, apoptotic: 13.51 ± 1.02%, necrotic: 12.29 ± 0.96%. n = 17-31

The ratio of necrotic cardiomyoblast cells in the untreated group was 48.09 ± 2.28%, which was significantly reduced in the hASC treated group (28.16 ± 1.94%), while it was significantly increased in the conditioned medium treated group (62.39 ± 1.89%) and did not change significantly in the hBMSC treated group (41.88 ± 2.46%). The ratio of the necrotic cardiomyoblast cells in the hASC treated group was significantly lower compared to the group treated with hBMSC (Figure 2A).

Examining the survival of the added therapeutic cells, we found that the majority of the added stem cells were alive both in the case of the hASCs (70.30 ± 2.35%) and hBMSCs (73.30 ± 1.92%) and there was no statistically significant difference between the two groups. There was no significant difference between the two groups neither in the apoptotic stem cells' ratio (hASC: 15.79 ± 1.22%, hBMSC: 13.51 ± 1.02%) nor in the necrotic stem cells' ratio (hASC: 13.41 ± 1.67%, hBMSC: 12.29 ± 0.96%) (Figure 2B).

The metabolic activity measurements strengthened our flow cytometric results. The metabolic activity of cells was significantly higher after the hASC (0.652 ± 0.089AU artificial units) and hBMSC treatments (0.607 ± 0.059 AU) than in the untreated group (0.065 ± 0.033AU). The conditioned medium treatment, although to a lesser extent, but also significantly increased metabolic activity (0.225 ± 0.013AU). There was no statistically significant difference between the hASC and hBMSC treated groups.

LDH release was significantly reduced compared to the untreated group (0.225 ± 0.006 AU) both in the hASC treated (0.148 ± 0.005 AU) and hBMSC treated groups (0.146 ± 0.004 AU).

4.3. Effects of PJ34 PARP inhibitor pretreatment of therapeutic cells in an in vitro model of ischemia-reperfusion

Our results show that the one hour treatment with the PARP inhibitor PJ34 did not increase in LDH release neither when used at a 10 μM concentration (-3.40 ± 0.16%) nor at 100 μM concentration level (-3.61 ± 0.27%) compared to the control group (-0.18 ± 0.36%).

Oxidative stress modeled by a two hours 400 μM H_2O_2 treatment significantly increased LDH release (35.14 ± 1.01%) compared to in the control group. One hour PJ34 PARP inhibitor treatment significantly reduced the effect of the H_2O_2 treatment both in the 10 μM (15.65 ± 0.95%) and 100 μM group (15.69 ± 0.54%) (Figure 3.).

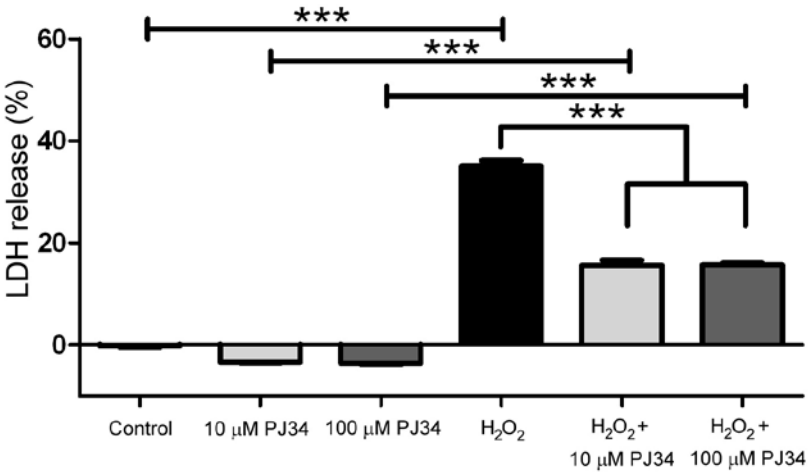

Figure 3. PJ34 cytotoxicity test and protection against oxidative stress. Control: -0.18 ± 0.36%, 10 μM PJ34: -3.40 ± 0.16%, 100 μM PJ34: -3.61 ± 0.27%, H_2O_2: 35.14 ± 1.01%, H_2O_2 + 10 μM PJ34: 15.65 ± 0.95%, H_2O_2 + 100 μM PJ34: 15.69 ± 0.54%. *** : $p < 0.001$, ANOVA, Newman-Keuls post hoc test, n = 6

In this series of experiments, the survival of the cardiomyoblasts undergoing ischemia-reperfusion injury was 36.44 ± 5.05% in the untreated group, which

did not change significantly in the group which received untreated H9c2 cells (42.81 ± 5.11%), however, it was significantly increased in the groups which received the 10 μM PJ34 PARP inhibitor pretreated therapeutic cells (52.07 ± 5.80%) or the 100 μM PJ34 PARP inhibitor pretreated therapeutic cells (54.95 ± 5.55%).

The apoptotic cells ratio between the cardiomyoblasts undergoing ischemia-reperfusion injury showed no significant differences between the experimental groups (I-R model: 19.94 ± 2.75%, H9c2: 20.23 ± 2.62%, H9c2 + 10 μM PJ34: 17.20 ± 2.42%; H9c2 + 100 μM PJ34: 20.05 ± 3.23%).

The ratio of the necrotic cells between the cardiomyoblasts undergoing ischemia-reperfusion injury was 43.64 ± 4.00% in the untreated group, which did not change significantly in the group which received untreated H9c2 cells (37.29 ± 4.55%), however, it reduced significantly in the groups which were receiving the 10 μM PJ34 PARP inhibitor pretreated therapeutic cells (30.18 ± 4.60%) and the 100 μM PJ34 PARP inhibitor pretreated therapeutic cells (25.52 ± 3.47%) (Figure 4A).

Examining the survival of the added therapeutic cells we found that the survival of the therapeutic cells receiving the PJ34 PARP inhibitor pretreatment was significantly higher compared to that of the untreated therapeutic cells (H9c2: 52.02 ± 5.01%, H9c2 + 10 μM PJ34: 63.38 ± 4.50%, H9c2 + 100 μM PJ34: 64.99 ± 3.47%).

The ratio of apoptotic cells between the therapeutic cells was not significantly different among groups (H9c2: 10.87 ± 1.12%, H9c2 + 10 μM PJ34: 9.22 ± 1.28%; H9c2 + 100μM PJ34: 10.18 ± 1.55%).

The PJ34 PARP inhibitor pretreatment significantly reduced necrosis of the therapeutic cells (H9c2: 37.23 ± 4.40%, H9c2 + 10 μM PJ34: 26.83 ± 3.49%, H9c2 + 100 μM PJ34: 24.96 ± 2.43%) (Figure 4B).

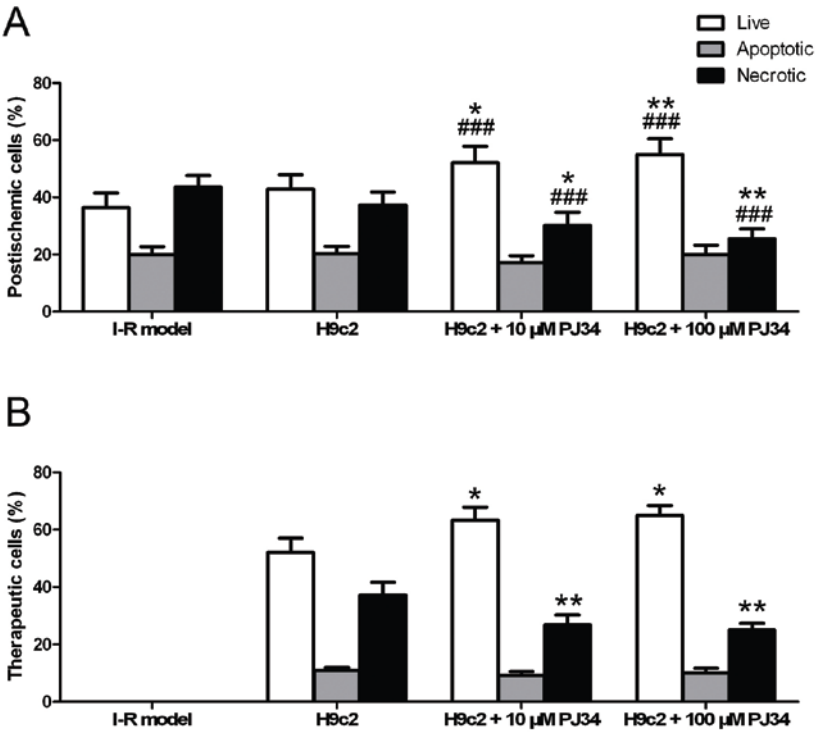

Figure 4. Flow cytometric analysis. A) The ratio of live, apoptotic and necrotic postischemic cells. I-R model: live: 36.44 ± 5.05%, apoptotic: 19.94 ± 2.75%, necrotic: 43.64 ± 4.00%; H9c2: live: 42.81 ± 5.11%, apoptotic: 20.23 ± 2.61%, necrotic: 37.29 ± 4.55%; H9c2 + 10 μM PJ34: Live: 52.07 ± 5.80%, apoptotic: 17.20 ± 2.42%, necrotic: 30.18 ± 4.60%; H9c2 + 100 μM PJ34: live: 54.95 ± 5.55%, apoptotic: 20.05 ± 3.23%, necrotic: 25.52 ± 3.47%, * P <0.05, **: p <0.01 vs. H9c2, ###: p <0.001 vs. I-R model, ANOVA, Newman-Keuls post hoc test, n = 18 B) The ratio of live, apoptotic and necrotic therapeutic cells. H9c2: live: 52.02 ± 5.01%, apoptotic: 10.87 ± 1.12%, necrotic: 37.23 ± 4.40%; H9c2 + 10 μM PJ34: Live: 63.38 ± 4.50%, apoptotic 9.22 ± 1.28%, necrotic: 26.83 ± 3.49%; H9c2 + 100 μM PJ34: live: 64.99 ± 3.47%, apoptotic: 10.18 ± 1.55%, necrotic: 24.96 ± 2.43%, * : p <0.05, ** : p <0.01 vs. H9c2, ANOVA, Newman-Keuls post hoc test, n = 18

5. Conclusions

Based on our results the bone marrow and adipose tissue derived mesenchymal stem cells improve the survival of damaged cardiomyoblasts in our in vitro ischemia-reperfusion model through direct effects and by the same extent. The survival of the two types of stem cell in the post-ischemic environment is also similar. However, the hASC treatment resulted in a significantly greater reduction in the necrosis of the injured cardiomyoblasts directing the damaged cells toward the apoptotic cell death, which may partly explain the greater efficiency of the ASC cells observed in vivo. The adipose tissue which is readily available and in large quantities is a promising source of autologous mesenchymal stem cells, and the hASCs may represent a real alternative to the hBMSCs for the treatment of the infarcted heart. Based on our further investigations PARP inhibitor pretreatment of the therapeutic cells increased their survival in post-ischemic conditions and enhanced their therapeutic effect on damaged cardiomyoblasts.

If our results are strengthened by further in vivo studies, it may become possible to increase the efficiency of cell-based therapies by the wider application of hASCs, and by using PARP inhibitors for the pretreatment of the therapeutic cells used.

6. List of publications

6.1. Publications on the subject of the thesis:

Szepes M, **Benkő Z**, Cselenyák A, Kompisch KM, Schumacher U, Lacza Z, Kiss L: Comparison of the direct effects of human adipose- and bone-marrow-derived stem cells on postischemic cardiomyoblasts in an in vitro simulated ischemia-reperfusion model.
Stem Cells Int. 2013;2013:178346. doi: 10.1155/2013/178346. Epub 2013 Jun 19. **IF: 2,806 - shared first authorship**

Szepes M, Janicsek Z, **Benkő Z**, Cselenyák A, Kiss L:
Pretreatment of therapeutic cells with poly(ADP-ribose) polymerase inhibitor enhances their efficacy in an in vitro model of cell-based therapy in myocardial infarct.
Int J Mol Med. 2013 Jan;31(1):26-32. doi: 10.3892/ijmm.2012.1186. **IF: 1,880**

6.2. Other peer-reviewed publications:

Dongó E, **Benkő Z**, Csizmazia Á, Marosi G, Grottke A, Jücker M, Schumacher U, Kiss L.: H2S preconditioning of human adipose tissue-derived stem cells increases their efficacy in an in vitro model of cell therapy for simulated ischemia.
Life Sciences, 2014, in press doi: 10.1016/j.lfs.2014.07.023 **(IF in 2013: 2,296) - shared first authorship**

Cselenyák A, **Benkő Z**, Szepes M, Kiss L, Lacza Z:
Stem cell transplantation in an in vitro simulated ischemia/reperfusion model.
J Vis Exp. 2011 Nov 5;(57):e3575. doi: 10.3791/3575.

Dongó E, Hornyák I, **Benkő Z**, Kiss L:
The cardioprotective potential of hydrogen sulfide in myocardial ischemia/reperfusion injury.
Acta Physiol Hung. 2011 Dec;98(4):369-81. doi: 10.1556/APhysiol.98.2011.4.1. Review. **IF: 0,821**

Cselenyák Attila, **Benkő Zsolt**, Szepes Mónika, Dr. Horváth Eszter Mária, Dr. Lacza Zsombor, Dr. Kiss Levente:
Az őssejtek szerepe a szívinfarktus kezelésében: in vitro kísérletes módszer a hatásmechanizmus vizsgálatára
Érbetegségek – A Magyar Angiológiai és Érsebészeti Társaság tudományos folyóirata, 2011/1 3-11. oldal

Dr. Kiss Levente, Dongó Eleni, Janicsek Zsófia, Szepes Mónika, **Benkő Zsolt**, Cselenyák Attila, Dr. Lacza Zsombor:
Őssejtterápia alkalmazásának eredményei perifériás artériás érbetegségben
Érbetegségek – A Magyar Angiológiai és Érsebészeti Társaság tudományos folyóirata, 2010/3 33-38. oldal