Summary

The aim of the present study was to evaluate the possible beneficial effects of implantation of laser-irradiated mesenchymal stem cells (MSCs) into the infarcted rat heart. MSCs were isolated from rat bone marrow and grown in culture. The cells were laser irradiated with Ga-Al-As laser (810 nm wavelength), labeled with 5-Bromo-2’deoxyuridine (BrdU), and then implanted into infarcted rat hearts. Non-irradiated cells were similarly labeled and acted as control. Hearts were excised three weeks later and cells were stained for BrdU and c-kit (Stem cell marker) immunoreactivity. Infarcted hearts that were implanted with laser-treated cells showed a significant reduction of 53% in infarct size and a 5-6.3-fold significant increase in cell density that reacted positively to c-kit. The findings of the present study provide the first evidence that LLLT can significantly increase survival and/or proliferation of MSCs post implantation into the ischemic/infarcted heart, followed by a marked reduction of scarring, and enhanced angiogenesis.

Introduction

Cardiac diseases are one of the leading causes of mortality in the western world. Recent findings have demonstrated the existence of stem cells within the adult mammalian heart.\textsuperscript{1,2} Despite these encouraging findings, the ability of the heart to regenerate is very limited, and can not compensate for the loss of cardiomyocytes post myocardial infarction (MI).

One approach in the attempt to overcome the limited capacity of the cardiomyocytes to proliferate after ischemia, is that of the implantation of
various cell types (skeletal muscle satellite cells, mesenchymal stem cells (MSCs) etc.) into the myocardium post MI. Despite the positive reports regarding experimental animal models, the studies in humans have shown mixed results. Recently, in three double-blind clinical trials on a total of 426 patients with ischemic heart failure, there was a minimal or no improvement of heart function up to six months post cell implantation in patients that received autologous MSCs as compared to saline.

Low-energy laser therapy (LLLT) has been found to modulate various biological processes. The beneficial effects of LLLT on the ischemic/infarcted heart have been previously demonstrated in several studies. This phenomenon was partially attributed to a significant elevation in the number of undamaged mitochondria, heat shock proteins, ATP content and angiogenesis in the ischemic zone of the laser-irradiated rat hearts as compared to non-irradiated rat hearts. It was also demonstrated recently that LLLT significantly promoted the growth of MSCs and cardiac stem cells in culture. The effect of LLLT on MSCs prior to their implantation into the infarcted heart or other ischemic organ has not yet been investigated. The aim of the present study was to investigate the advantages of low-level laser treated MSCs for use as cell therapy. The rat infarcted heart was used as an experimental model for this phenomenon in this study.

Materials and Methods

A total of 38 Charles River mature (2-3 months old) male rats weighing 200-250 gr were used in this study: ten rats were used for MSCs isolation and 28 for cell implantation. Isolation of MSCs was performed essentially as described by Davani et al. Following isolation of MSCs, as described previously cells were labeled with 0.06% 5-Bromo-2′-deoxyuridine (BrdU). The BrdU was added to the cell culture every two days for two weeks prior to implantation in order to label the nuclei of the implanted cells. About 90% of the isolated cells demonstrated c-kit (a marker of MSCs) positive staining in culture. A diode (Ga-Al-As) laser, wavelength 804 nm with a tunable power output of maximum 400 mW was used (Lasotronic Inc., Zug, Switzerland). Laser irradiation applied to the tissue cell cultures was performed as described previously. Control cells underwent the same procedure as the laser-irradiated cells but the laser was set to “off” position. Control and laser-irradiated plates were chosen at random. MI was induced in 28 rats as described previously. The BrdU labeled isolated MSCs were injected, 30 min after induction of MI into two sites, one near the infarction area and the other in the lower part of the left ventricle. The hearts were excised and two horizontal serial cross-slices (2 mm thick) were prepared. One slice was taken from the infarcted area (1 mm distal to the point of ligation) for electrophoresis (immunoblotting), and the second serial slice was taken from the area (distal to the first slice) for determination of infarct size and immunohistochemistry. Histology and infarct size were determined using Masson’s trichrom stained histological sections as described previously. In order to detect the
implanted MSCs (labeled with BrdU prior to implantation, as described above) BrdU immunostaining kit was used. The total number of MSCs in the heart was determined using anti-c-kit. The SigmaStat 2.0 software was used for statistical analysis. Tests were performed for normality distribution, followed by ANOVA test.

Results

Implantation of non-laser-treated MSCs to the infarcted heart caused a significant (p<0.012) 50% reduction in infarct size as compared to the injection of their vehicle (saline) alone (Fig. 1). Figure 1 also demonstrates that implantation of laser-irradiated MSCs caused a significant (p<0.006) reduction of 53% in infarct size three weeks post MI, compared to infarct size in hearts with implanted MSCs that were not laser-irradiated prior to implantation (control). A significant (p<0.004) reduction of 76% of infarct size was also measured in hearts that were implanted with laser-irradiated cells as compared to saline injected hearts (Fig. 1).

Anti-c-kit antibody was used for detection of MSCs in the infarcted hearts. Cells positively immunoreacting to c-kit were rarely found in the infarcted myocardium that was injected with saline (Fig. 2). The results (Fig. 2) demonstrate a significant (p=0.046) increase (6.3-fold) in the density of c-kit immunopositive cells in the infarcted area in hearts implanted with laser-treated MSCs compared to control.

Conclusions

The present study clearly demonstrates that application of LLLT to MSCs

Figure 1. Effect of saline injection and MSCs implantation into the infarcted heart on infarct size in the infarcted rat heart. Infarcted hearts were injected (30 minutes post infarction) with saline (open columns) or MSCs (dashed columns) or MSCs that were laser-treated prior to implantation (solid columns). Infarct size was expressed as percentage of left ventricle area. Each column represents mean ± SEM of 8-10 rats.
prior to their implantation into the infarcted heart has beneficial effects on the recovery of the ischemic heart. Implantation of MSCs without laser pre-treatment also resulted in a reduction in infarct size in comparison to injection of their vehicle (saline) alone. This phenomenon is in accordance with previous studies, in experimental animals, that showed improvement in functional performance of the infarcted heart following implantation of various cell types (usually myogenic cells or stem cells). However, the laser pre-treatment of the implanted cells demonstrated additional beneficial effects beyond those achieved through implantation of untreated MSCs. These effects of the laser treatment were evident in a marked reduction of infarct size. In addition, the results of this study demonstrate a 6.3-fold significant increase in c-kit immuno-positive cells in the infarcted myocardium of hearts injected with laser-treated MSCs over the non-treated MSCs. This indicates that the implanted cells that were laser-treated prior to implantation proliferated and/or survived to a much higher extent post implantation as compared to the non-laser-treated cells. Indeed, we have previously shown that application of the laser to the ischemic heart and skeletal muscles caused a significant increase in the expression of heat shock proteins (HSP-70i), which are known to be cytoprotective. 12 In this study we did not investigate the migratory pathways and differentiation of the implanted cells. The results nonetheless indicate that cell density of c-kit positive cells demonstrated an 8-fold increase in the infarcted area as compared to their density in the entire left ventricle in the hearts that were injected with non-laser-treated MSCs. Thus it can be postulated that, following implantation, the MSCs homed specifically in the infarcted area from the entire left ventricle area. In the hearts that were injected with the laser-treated cells the c-kit positive cell density was 22-fold higher in the infarcted area as
compared to the entire left ventricle, while in the hearts injected with nontreated cells this value was only 8-fold higher. Thus, it can be postulated that the laser treatment caused an increase in migratory capabilities of the MSCs and their affinity to migrate to the infarcted area compared to non-treated MSCs. These results corroborate with those of a recent study on stroked rats, in which more cells (stem cells) in the subventricular zone in the brain immunoreacted to doublecortin (a marker indicating the migratory capacity of neurons) in the laser-treated rats relative to the non-treated ones.13

The results of the present study have implications in the field of cell therapy in general as well as possible clinical application. Many previous publications have addressed the issue of survival of cells post implantation to organs in general. This subject is of prime significance in the developing field of cell and tissue engineering. Thus the increased survival of implanted cells as demonstrated in this study following LLLT has a significant role in the field of cell therapy. The possible clinical application of the present study arises from the extensive clinical trials that were summarized recently14 on the safety and limited beneficial effects of the use of MSCs implantation to the human myocardium post MI. These studies indicate that the technology appears to be safe and even demonstrates some improvement of heart function in patients that received implantation of MSCs. The possibility of applying the laser treatment to MSCs prior to their implantation to human hearts in order to achieve a higher improvement in heart function (beyond what is currently achieved without the laser pretreatment) is feasible. In addition the application of LLLT in vivo to heart and brain of experimental animals,7,9,12,13 as well as to humans post acute stroke,15 seems to be safe and without side effects.

References
9. Tuby H., Maltz L., and Oron U. Modulations of VEGF and iNOS in the rat heart by low level laser therapy are associated with cardioprotection and enhanced