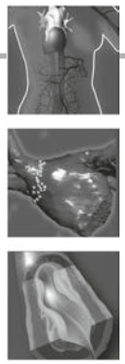

Light therapy and stem cells: a therapeutic intervention of the future?

"...a novel approach of applying LLLT to autologous bone marrow of infarcted rats in order to induce the proliferation of stem cells that are consequently recruited to the ischemic heart, leading to a marked beneficial effect post-MI."

KEYWORDS: bone marrow ■ low-level laser therapy ■ myocardial infarction ■ stem cells

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The mammalian heart has a very limited capacity to regenerate following damage or an acute ischemic event, such as myocardial infarction (MI), due to the very low level of cardiomyocyte proliferation and the limited number of cells expressing stemcell marker proteins. Stem-cell-based therapy was suggested as a potential solution to the above situation. In recent years, cell-based therapy for cardiac repair has undergone a rapid transition from basic science research to clinical reality [1–3]. The general outcome of the clinical trials was that the procedures and long-term outcome post-stem-cell implantation to the heart, via the coronary arteries, are safe. However, improvement in long-term functional performance of the heart was either not achieved or was marginal [1–3].

There are several central issues pertaining to the use of cell implantation in stemcell therapy for cardiac repair: the number of implanted stem cells has to be high, since there is massive cell death following implantation or injection of cells into the heart or the blood circulation. Another central issue in stemcell implantation for cardiac repair is the creation of a receptive cell environment in the infarcted heart. Several factors (e.g., inhibition of inflammation and apoptosis, secretion of cell growth factors) are necessary for optimal cell implantation [4]. The injected cells may have to migrate from the circulating blood to the heart or within the heart to an appropriate area in the heart. They can then remain active and secrete growth factors, exerting a paracrine effect on the ischemic tissue [5]. Alternatively, they may stimulate the small population of stem cells in the ischemic heart to proliferate and differentiate to enhance cardiac repair post-MI [6]. Another issue concerns the timing of injection of the stem cells to the infarcted heart and effect of MI (inflammatory phase) on the bone marrow (BM) [7].

Oron *et al.* were trying to overcome some of the above mentioned issues of stem cell therapy for cardiac repair by using light stimulation therapy (low-level laser therapy [LLLT]) to stem cells in the BM. LLLT has been found to modulate various biological processes [8], such as increasing mitochondrial respiration and ATP synthesis, facilitating wound healing, and promoting the process of skeletal muscle regeneration and angiogenesis [9]. In an experimental model of the infarcted heart in rats and dogs, it was demonstrated that LLLT application directly to the infarcted area in the heart at optimal power parameters significantly reduced infarct size (scar tissue formation) [10]. This phenomenon was partially attributed to a significant elevation in ATP content, heat shock proteins, VEGF and angiogenesis in the ischemic zone of the laser-irradiated rats, as compared with non-irradiated rats [10,11]. The mechanism associated with the photobiostimulation by LLLT is not yet fully understood [8]. There is evidence that cytochrome C oxidase, and perhaps also plasma membrane, in cells function as a photoacceptor of the photons, and thereafter a cascade of events occure in the mitochondria leading to effects on various processes, such as ATP production and upregulation of VEGF among others.

The effect of photobiostimulation on stem cells or progenitor cells has not been extensively studied [12–14]. We have previously shown that laser application to the mesenchymal stem cells isolated from BM or cardiac stem cells causes a significant increase in their proliferation *in vitro* [14]. Recently, based on our previous studies that showed an increase in cytoprotective effect on the ischemic heart following LLLT, we took a new approach regarding the application of laser irradiation to stem cells prior to their

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implantation to the infarcted heart as a cell therapy for heart repair [15]. In that study we demonstrated that mesenchymal stem cells (MSCs) that were laser-treated prior to their implantation to the rat infarcted heart caused a significant reduction in infarct size, compared with MSCs that were injected to the heart without prior laser treatment. This phenomenon was also associated with significant elevation of VEGF in the myocardium of the rats that received the laser-treated MSCs. In our recent study we addressed the possibility of recruiting autologous stem cells stimulated by LLLT in the BM to the infarcted heart [16]. We found that when LLLT was applied *in vivo* to the BM and MSCs were isolated from that BM 3 and 6 weeks later and grown *in vitro*, they grew at a higher rate of proliferation relative to MSCs isolated from non-laser-treated BM. This indicates that the MSCs, when in the BM, following LLLT application *in vivo* can be induced to proliferate to a higher rate than non-treated MSCs. Furthermore, laser application to the BM (at about 20 min post-MI) caused a marked and significant decrease (79%) in infarct size 3 weeks post-MI. This extent of infarct size reduction was even more effective in reducing scarring than that of laser application directly to the infarcted heart, as also found in our previous studies with infarcted rat and dog hearts [10]. Even when laser was applied 4 h post-MI to the BM of infarcted rats, a marked (52 and 42%) and significant ($p < 0.01$) reduction in the infarcted area was observed in the laser-treated rats compared with the control. We also found a significantly higher density of c-kit$^+$ (marker of MSCs) cells in the myocardium of laser-treated rats relative to non-treated rats post-MI. Moreover, it was demonstrated in this study that c-kit$^+$ cells post-laser application to the BM of MI-induced rats homed specifically in on the infarcted heart and not in on uninjured organs (i.e., liver, kidney) in the same rat [16]. It can be hypothesized that the increased number of c-kit$^+$ cells found in the myocardium originated from proliferating MSCs in the BM that had migrate to the circulating blood and homed into the infarcted heart.

Another finding from our recent studies is that of the preferred homing of the recruited or endogenous c-kit$^+$ cells on the infarcted area, rather than random deposition throughout the left ventricle. Indeed, at 3 weeks post-MI the density of c-kit$^+$ cells in the infarcted area was 27-fold higher in the rats whose BM had been treated with LLLT as compared with control rats. Similarly, Hatzistergos *et al.* found that endogenous c-kit$^+$ cardiac stem cells increased by 20-fold in the porcine infarcted heart as compared with control following transcardial injection of BM-derived MSCs [6].

Our recent study also has direct clinical relevance. The laser can be applied noninvasively (or invasively by inserting a fiber optic to the iliac crest in obese patients) to the bone marrow of the pelvic girdle, tibia or other parts of the skeleton containing BM up to 4 h post-MI. This time interval post-MI is a reasonable therapeutic window for the laser treatment. The novel approach presented in this study, using stem cells for cell therapy directly to the infarcted heart, avoids the need to isolate millions of stem cells, to grow them *in vitro* and to inject them back into the patients. Mobilization of endothelial progenitor cells into the circulation and their increased number in the blood of patients post-MI, as compared with non-MI patients, has been reported in the past [17]. It can be postulated that the body's 'attempt' to mobilize various progenitor cells via the blood system to the infarcted heart may be a normal response post-MI in patients. However, this response does not seem to cause attenuation of the scarring process post-MI. Thus, it can be postulated that the novel approach presented in our recent study [16], of the stimulation of stem cells in order to increase their number in the blood, and eventually in the infarcted heart, may also attenuate the scarring process in human patients post-MI.

The rationale behind the attempt to use LLLT to induce the 'crude' BM in the bone was, and still is, that one cannot significantly affect the complex process post-MI or ischemic injury to the kidney with a single type of stem cell. The native BM is known for its many types and subtypes of stem cells, which are defined by their reactivity to various antibodies.

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The BM also contains many progenitor cells (i.e., monocytes) that can further differentiate, for example to macrophages. Macrophages have been shown recently to play a crucial role in the scarring process post-MI. Thus, LLLT may induce concomitantly in the BM various types of cells that will increase in number in the blood circulation following their enhanced

proliferation in the BM. These cells will probably, eventually, be able to (under the right circumstances), home in on the ischemic zone in the ischemic organ (i.e., heart to a certain extent). The above hypothesis of course still requires proof and demonstration via further experimental studies. Nevertheless, it may be speculated that by controlling the recruitment or homing of stem cells from the circulation to the ischemic organ, one avoids the phenomenon of the massive the death of stem cells that characterizes the process of cell implantation to the ischemic heart. It is true that delivery of stem cells from BM to the heart via the blood may not deliver millions of cells at the particular time when they are needed, as is the case in stem-cell implantation. However, it has been demonstrated in experimental animals that an increase in the number of the implanted cells does not cause any correlated increase in heart function.

In conclusion, we have demonstrated a novel approach of applying LLLT to autologous bone marrow of infarcted rats in order to induce the proliferation of stem cells that are consequently recruited to the ischemic heart, leading to a marked beneficial effect post-MI. The possibility that this approach can also be applied to other ischemic/injured organs or organs undergoing degenerative processes (i.e., neurodegenerative diseases), with consequent beneficial effects, cannot be ruled out in the future.

Financial & competing interests disclosure

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