Regenerative capacity following injury or an ischemic event is confined to non mammalian vertebrates. Mammals have a limited capacity to restore organs following injury to organs like the liver and skeletal muscles but practically no ability to regenerate organs like the heart or brain following an ischemic event or injury. We tried a new approach in cell based therapy to improve regeneration in various organs following ischemic injury. Low-level laser therapy (LLLT) which has photobiostimulating effects on cells was delivered to autologous bone marrow (BM) that is enriched with stem cells and various progenitor cells, in order to induce the cells in the BM for the benefit of the injured /ischemic organs. In a model of induced myocardial infarction (MI) in rats laser application to the BM caused a marked and significant decrease (79%) in infarct size (scarring) 3 weeks post-MI. It was also found that a significantly higher density of c-kit positive cells (a marker of mesenchymal stem cells) in the myocardium of laser-treated rats relative to non-treated rat’s post-MI. The novel approach presented in this study, of the use of stem cells for cell therapy to the infracted heart, avoids the need to isolate millions of stem cells, to grow them in vitro and to inject them back into the patient. In the same line of rationale we tried to find whether LLLT to the BM could be beneficial also to kidney impairment after ischemic reperfusion injury (IRI) to the rat kidney. C-kit positive cell density in kidneys post-IRI and laser-treatment was significantly (p<0.05) 2.4-fold higher compared to the non laser treated group. Creatinine, blood urea nitrogen, and cystatin-C levels were significantly lower in the laser-treated rats as compared to non-treated ones. The effect of LLLT delivery to BM was also tested on Alzheimer’s disease (AD) mice in their late stage of the disease. Mice were given multiple (every 10 days) LLLT to BM from age 4 to 6 months. It was found that in the treated AD-mice neurological tests (Fear and Cognitive tests) revealed a significantly (p<0.05) better neurological performance and cognitive capacity compared to the non-treated AD mice. Furthermore, concomitantly with the improved neurological performance, β-amyloid density in the hippocampal region of the brains was revealed to be significantly less in the laser-treated mice as compared to control. In conclusion, a novel approach, of applying LLLT to autologous BM in order to induce stem cells that are consequently recruited to the injured/ischemic organ leading to a marked beneficial effect post-ischemic event or degenerative process is presented. This approach is novel in the respect that it is stimulating the patient’s own abilities to initiate a regenerative response in an organ by the utilization of light. 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.
paracrine effect on the ischemic tissue [5]. Alternatively, they may stimulate the small population of stem cells in the ischemic organ (such as the heart), to proliferate and differentiate so as to enhance cardiac repair post-MI [6]. Another issue is the timing of injection of the stem cells to the infarcted heart and effect of MI (inflammatory phase) on the BM [7]. Photobiostimulation of cells in the bone marrow (BM), that is enriched with various progenitor cells, by low level laser therapy (LLLT) may suggest a new approach that may overcome some of the above limitations. This new approach will be discussed in the present mini review [8-11].

LOW LEVEL LASER THERAPY FOR THE ISCHEMIC HEART

In general LLLT has been found to modulate various biological processes, such as increasing mitochondrial respiration and ATP synthesis, facilitating wound healing, and promoting the process of skeletal muscle regeneration and angiogenesis [12,13]. It was previously shown that LLLT can enhance skeletal muscle regeneration following partial excision in the rat hind limb muscles when the laser was delivered directly to the injured organ multiple times (for 2 min each time) following injury [14]. This phenomenon was even more prominent following cold injury to the frog skeletal muscles indicating that enhancement of regeneration by LLLT is probably a general phenomenon in vertebrates and maybe more effective in cold blooded animals which innately have a lower metabolic rate in their cells [13-15].

In an experimental model of the infarcted heart in rats and dogs, it was demonstrated that LLLT (Diode –Ga-Al-As 810nm at a power density of 5 mW/cm² for 120sec duration of laser exposure comprising 0.6 J/cm²), application directly to the infarcted area in the heart at optimal power parameters significantly reduces infarct size (scar tissue formation) [16,17]. This phenomenon was partially attributed to a significant elevation in ATP content, heart shock proteins, vascular endothelial growth factor (VEGF), and angiogenesis in the ischemic zone of the laser-irradiated rats, as compared to non-irradiated rats [16,17]. The mechanism associated with the photobiostimulation by LLLT is not yet clearly understood [12]. There is evidence that cytochrome c oxidase and perhaps also plasma membranes in cells function as photoacceptors of the photons, and thereafter a cascade of events occurs in the mitochondria, leading to effects on various processes like ATP production, up-regulation of VEGF, etc [12].

The effect of photobiostimulation on stem cells or progenitor cells has not been extensively studied [18-21]. It was previously shown that laser application (Diode laser at 50mW/cm² for 100sec, energy density 0.5 mW/cm²) to the mesenchymal stem cells isolated from bone marrow or cardiac stem cells causes a significant increase in their proliferation in vitro [20]. Based on previous studies that showed an increase in cytoprotective effect on the ischemic heart following LLLT, a new approach was taken to apply laser irradiation to stem cells grown in culture prior to their implantation to the infarcted heart as a cell therapy for heart repair [21]. In that study it was demonstrated that MSCs that were laser treated prior to their implantation to the rat infarcted heart caused a significant reduction in infarct size as compared to MSCs that were injected to the heart without prior laser treatment. This phenomenon was also associated with significant elevation of vascular endothelial growth factor (VEGF) in the myocardium of the rats that received the laser-treated MSCs. In a recent study [8] the possibility of recruiting autologous stem cells stimulated by LLLT in the BM to the infarcted heart was addressed. 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. The BM also contains many progenitor cells (i.e. monocytes) that can further differentiate, for example to macrophages. Macrophages have been shown recently to have 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, and to a certain extent and under certain circumstances, home in on the ischemic zone in the ischemic organ (heart, kidney etc.). In this study [8] it was 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 indicated 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 (Diode laser 808nm at power density of 10mW/cm² for 100 sec comprising 1J/cm² energy density) 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 previous studies with infarcted rat and dog hearts [10]. Even when laser was applied 4 hours post-MI to the BM of infarcted rats, a marked and significant reduction in the infarcted area was observed in the laser-treated rats compared to control. We also found a significantly higher density of c-kit+ (a marker of MSCs) cells in the myocardium of laser-treated rats relative to non-treated rat’s 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 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 came from proliferating MSCs in the BM that had migrated to the circulating blood and homed onto the infarcted heart. Another finding of this study was that of the preferred homing of the recruited or endogenous c-kit+ cells in on the infarcted area, rather than their random deposition throughout the left ventricle in the heart. 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 to control rats. Similarly, Hatzistergos et al. [6] found that endogenous c-kit+ cardiac stem cells increased by 20-fold in the porcine infarcted heart as compared to control following transcatheter injection of BM-derived MSCs.

The results of application of LLLT to autologous BM of infarcted rats as described above study [8] also have direct clinical relevance. The laser can be applied non-invasively (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 hours post-MI. This time interval post-MI is a reasonable therapeutic window for the laser treatment. The novel approach presented in this study, of the use of stem cells for cell therapy 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 patient’s post-MI, as compared to non-MI patients, has been reported [22]. 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 a recent study [8], 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 subject’s post-MI.

LOW LEVEL LASER THERAPY TO THE BONE MARROW FOR THE BENEFIT OF IMPAIRED KIDNEY

In the same line of rationale we tried to find whether LLLT to the BM could be beneficial also to kidney impairment after ischemic reperfusion injury. Acute renal failure has a 50-80% mortality rate with limited treatment options. The rat model of moderate and acute ischemia-reperfusion was used. Injury to the kidneys was induced by excision of the left kidney and 60 min of IRI to the right kidney in each rat. Rats were then divided randomly into 2 groups, of non laser treated and laser-treated. LLLT was applied to the BM 10 min and 24 hrs post-IRI and rats were sacrificed 4 days post-IRI. Blood was collected before sacrifice for determination of blood markers for kidney function. Histological evaluation of kidney sections revealed a reduction in dilatation of the renal tubules, restored structural integrity of the renal tubules, and a significant reduction of 66% of pathological score in the laser-treated rats as compared to the non-laser-treated ones. C-kit positive cell density in kidneys post-IRI laser-treatment was significantly 2.4-fold higher compared to the non laser treated group. Creatinine, blood urea nitrogen, and cystatin-C levels were significantly lower in the laser-treated rats as compared to the non-laser-treated ones. It is concluded that LLLT application to the BM causes a significant increase in the density of stem cells in the kidneys post-IRI, probably by induction of stem cells in the BM, which subsequently migrate and home in on the injured kidney. Consequently, a significant reduction in pathological features and improved kidney function (as revealed from blood tests) post-IRI is evident.

LOW LEVEL LASER THERAPY TO THE BONE MARROW FOR THE BENEFIT NEURODEGENERATIVE DISEASE

The approach that LLLT to the BM could be beneficial for neurodegenerative diseases was also examined by us recently. Bone-marrow (BM) stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia. It has also been suggested that blood-derived microglia, and not their resident counterparts, have the ability to eliminate amyloid deposits by a cell-specific phagocytic mechanism. Defining efficient ways by which to stimulate BM stem cells to migrate to the brain might thus offer a crucial step in stem-cell therapy in Alzheimer’s disease (AD). It therefore postulate in a recent study [23] that LLLT to the tibia bone of the hind-leg in an AD animal model showing profound neuronal death in the brain, will have two main beneficial effects by activating BM derived microglia towards clearance of the neurotoxic a- bet oligomers and fibrils in addition to induction of neurogenesis towards neuroprotection. Mesenchymal stem cells (MSCs) isolated from BM of laser-treated and non-laser-treated mature rats were grown in culture. ß-amyloids that are over expressed in the brains of AD experimental animals or humans were added to the medium. The extent of ß-amyloid degradation by the MSCs was determined. It was found that the laser-treated MSCs degraded the ß-amyloids to a significantly higher extent than the non-laser-treated MSCs. In a concomitant experiment, AD mice were given multiple (every 10 days) LLLT to BM from age 4 to 6 months. In this mice model, at 4 months of age the mice show a well-established memory loss and accumulation of ß-amyloids in the brain. Non-laser, sham-treated, AD-mice served as control. It was found that in the treated AD-mice (laser-treated to the BM) neurological tests (Fear and Cognitive tests) revealed a significantly better neurological performance compared to the non-treated AD mice. Importantly, in the cognitive test the laser-treated mice improved to the level of intact (wild-type) mice. Furthermore, concomitantly with the improved neurological performance, ß-amyloid density in the hippocampal region of the brains was revealed to be significantly less in the laser-treated mice as compared to control. It was concluded that LLLT to the BM of AD mice most probably activates macrophagic activity in cells in the BM (microglial-like cells), which subsequently migrate from the BM to the circulating blood and enter the brain. There, they may cause the degradation of ß-amyloids, with a consequent significant improvement in cognitive function of the brain in the AD mice.

LIMITATION OF LIGHT THERAPY TO BONE MARROW FOR REGENERATIVE MEDICINE

CONCLUDING REMARKS

The current novel approaches presented in this review have also limitation when clinical application will be considered. The technology has yet to be tested in large animal model for its efficacy. Many degenerative diseases and ischemic conditions (like after MI, stroke, kidney diseases etc.) occur in old age when the BM becomes more fatty and progenitor cells and stem cells do not populate the BM to the same extent as in young human subjects or experimental animals. This phenomenon may affect the efficacy of the approach to stimulate by light the bone marrow to enhance regeneration.

In conclusion, we have demonstrated here a novel approach, of applying LLLT to autologous BM in order to induce stem cells that are consequently recruited to the injured/ischemic organ leading to a marked beneficial effects post-ischemic event in various organs and degenerative stages. This approach is novel in the respect that it is stimulating the patient’s own abilities to initiate a regenerative response in an organ by the utilization of light. Importantly, this approach may be of significance in
those organs like the heart and brain in mammals that has very limited resources of progenitor cells in the organ itself. Indeed in the infarcted heart the reduction of scarring was to a higher extent when LLLT was applied to the BM rather than directly to the heart [8]. It should also be noted that the induction of various cells in the BM by LLLT may deliver several types of cells to the ischemic organ that will home there and act in concert to attenuate processes post ischemia and enhance regeneration. This was evident in the study of LLLT to BM in AD mice [23] when microglial-like cells induced by the LLLT contributed to the beneficial effect on the cognitive state of these mice. 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.

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