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Steven Barbour
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Dear Steven Barbour,

Sorry for the tardiness in sending this information. I have also included 1st page of Endogenous Stem Cell presentation. The stem cell paper is still proprietary and I would like to keep it between us. Hopefully you see the potential of using this combination in treating many diverse problems. I plan on upgrading our laser to your newest model to accurately perform research projects. Please feel free to contact me with any questions.

Sincerely,

Alan S. Lichtbroun, MD
Low-level Laser Irradiation Stimulate Proliferation and Differentiation of Human Stem Cells

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Low-level laser therapy (LLLT) has been applied clinically for treating musculoskeletal pain, wound healing, acute and chronic inflammation. Moreover, many studies have demonstrated positive biostimulatory effects of LLLT on cells. LLLT can stimulate and promote the migration and proliferation of various cells [1,2,3]. The proliferation, growth factor secretion and differentiation of mesenchymal stem cells is also enhanced by LLLT [4,5,6]. Until now the mechanism of LLLT for cell proliferation remains unclear. Several possible mechanisms and related signaling pathways have recently been found. LLLT can regulate mitochondrial signaling, activate calcium channels, and phosphorylate certain growth factors. Cell cycle associated genes in mesenchymal stem cells are increased after LLLT treatment in a time dependent manner. Microarray assays reveal subsets of miRNAs to be differentially regulated and these dynamic changes are confirmed by quantitative real-time polymerase chain reaction. miRNA-193 was the most highly upregulated miRNA, and the change in it was related to the level of proliferation [8]. LLLT can also affect the differentiation of these cells. We will present an example of gallium aluminum arsenide (GaAlAs) laser irradiation (810nm) to induce bone marrow stem cells to osteoblasts and neurons in the range of 2-6 J/cm^2 [9]. LLLT has also been widely applied to retard the inflammatory environment. It has recently been shown that human stem cells express Toll-like receptors 1,3,4 and 6 and lipopolysaccharide significantly included pro-inflammatory cytokines (COX-2, IL-1beta, Interleukin-6 and Interleukin-8). LLLT markedly inhibits the pro-inflammatory cytokine expression at an optimal dose of 8 J/cm^2. The inhibitory effect triggered by LLLT might
occur through an increase in the intracellular level of cyclic AMP, which acts to
down-regulate nuclear factor B transcriptional activity [10].

We will present an example of LLLT effect on mesenchymal stem cells to
accelerate skin regeneration in athymic mice. The LLLT enhances wound healing
including neovascularization and regeneration of skin appendages compared to the
control mesenchymal stem cells only group. In addition to the hair follicles and
sebaceous glands, some cytokeratin positive-ASCs were observed in regenerated
epidermis. The survival of these stem cells was also increased due to the decrease
apoptosis in the wound bed of the stem cells. The secretion of growth factors
such as Vascular Endothelial Growth Factor (VEGF) and basic fibroblast growth
factor was also increased. VEGF is the most effective growth factor for
angiogenesis, BFGF is an important growth in wound healing because it affects
migration of fibroblasts and matrix deposition [8b]. Several recent studies report
a significant decline in the MSC number in skin wound bed, bone defect or
infarcted myocardium within the initial two week [9b,10b]. This study shows an
increase in ASC number with LLLT compared to a control at twenty-one days [11].
This suggests that LLLT enhanced the survival of adult stem cells by the inhibition
of apoptosis. More VEGF and bFGF-positive ASCs were observed in the
regenerated dermis after LLLT treatment. These data suggest that LLLT enhanced
not only their survival but also the functionality of the transplanted ASCs in the
wound bed. In addition LLLT increases the gene expression and the release of
several growth factors such as nerve growth factors from stem cells via increases
in the mitochondrial membrane potential and ATP and cAMP potentials [12]. We
plan on using the above abilities of LLLT to potentiate the therapeutic potential of
endogenous stem cells in musculoskeletal repair and healing. Future studies will
also involve amelioration of diabetes, autoimmune thyroid disease, toxic induced
liver cirrhosis and coronary disease.
The Therapeutic Potential of Stimulating Endogenous Stem Cell Mobilization

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ABSTRACT

The past few years have seen extensive interest in treatment of various diseases with adult stem cells (ASC). This paper will not discuss umbilical cord, adipose tissue derived stem cells, but rather peripheral blood stem cells. We will demonstrate that one of the natural roles of stem cells is to participate in tissue repair both due to injury or degenerative disease. The clinical relevance of mobilizing endogenous bone marrow derived stem cells would be to increase the number migrating into affected cells and contribute to tissue repair.

SIGNALING FOR MOBILIZATION

The most common compound known to stimulate BMSC mobilization is Granulocyte-Colony Stimulating Factor discovered in 1985 by Welt (Welte K, Platzer E, Lu L, Gabrilove JL, Levi E, Mertelsmann R, and Moore MA., 1985). GCSF is a cytokine that stimulates the proliferation, differentiation and function of neutrophil precursors and BMSC mobilization making it a tool in stem cell apheresis (Gordon MK, Sher D, Karrison T, Kebrae P, Chuang K, Zhang Y, McDonnell D, Artz A, Godley L, Odenike O, Rich E, Michaelis L, Thirman MJ, Wickrem a A, van Besien K, Larson RA, Stock W., 2008). As an example a few hours after an acute MI the cardiac tissue releases GCSF. This increases the number of BMSC which peaks 4-7 days after AMI (Leone AM, Rutella S, Bonanno G, Contemi AM, de Ritis DG, Giannico MB, Rebuzzi AG, Leone G, Crea F., 2006; Leone AM, Galiuto L, Garramone B, Rutella S, Giannico MB, Brugaletta S, Perfetti M, Liuzzo G, Porto I, Burzotta F, Niccoli G, Blasucci LM, Leone G, Rebuzzi AG, Crea F., 2007). Other chemokines such as interleukine-8 (IL-8), Stromal-Derived Factor-1 (SDF-1), Stem Cell Factor (SCF) and VEGF have been shown to trigger BMSC mobilization (Fukuda S, Bian H, King AG, Pelus LM., 2007). CVA also triggers the number of PBSC which tripled within 7 days after a stroke and is correlated with the functional recovery of the patients (Hennemann B, Ickenstein G, Sauerbruch S, Luecke K, Haas S, Horn M, Andreesen R, Bogdahn U, Winkler J., 2008; Paczkowska E, Larysz B, Rzeuski R, Karbicka A, Jałowiński R, Kornacewicz-Jach Z, Ratajczak MZ, Machaliński B., 2005). This is not seen after thrombolysis, but lingering injury leads to stem cell mobilization. Finally injury of skin, bone and joints trigger BMSC migration into injured tissue. After severe burns PBMC increased up to 9-fold (Mansilla E, Marin
revealed after 4-13 months a significant number of Y-chromosome hepatocytes. In one patient who died hepatitis C after a liver transplant, 43% of the transplanted liver was made up of Y chromosomes (Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, Krause DS., 2000). Similar observation are seen in men after cardiac transplants from female donors where on average 1-15% of cardiomyocytes were Y-chromosome positive (Lafayette MA, Myerson D, Saffitz JE, Murry CE., 2002). In one patient who died of cardiac rejection, 29% were Y-chromosome positive in areas of high cardiac repair. This is also seen in men who receive lung transplants from female donors. Similar observations are seen in skin injury models. For example, irradiated mice were transplanted with GFP positive stem cells. In 48 hours there were GFP-positive in the deep layers of the skin, while in 4 weeks these cells were composing the structure of the healed skin including blood vessels, sebaceous glands, and rare muscle fibers and hair follicles that were not seen in the control animals who received similar stem cells but no punch biopsy (Abedi M, Greer DA, Colvin GA, Demers DA, Dooner MS, Harpel JA, Pimentel J, Menon MK and Quesenberry PJ, 2004).

SDF-1 have been shown to stimulate proliferation and survival of stem cells Broxmeyer HE, Kohli L, Kim CH, Lee Y, Mantel C, Cooper S, Hangoc G, Shaheen M, Li X, Clapp DW., 2003). There is also a paracrine effect of BMSC which leads to an increase in concentration of IL-10, intereleukin-1beta and TNF-alpha. For example this contributes to neovascularization and reduction of cardiac infarct size in acute MI (Burchfield JS, Iwasaki M, Koyanagi M, Urbich C, Rosenthal N, Zeiher AM, Dimmeler S., 2008; Mirotseu M, Jayawardena TM, Schmeckpeper J, Gnech M, Dzau VJ., 2011).

There is a link between circulating stem cells and predictors of disease progression. This has been shown in cardiac disease (Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Böhm M, Nickenig G., 2005), arthritis (Herbrig K, Haensel S, Oelschlaegel U, Pistorosch F, Foerster S, Passauer J., 2006), kidney failure (Herbrig K, Pistorosch F, Oelschlaegel U, Wichmann G, Wagner A, Foerster S, Richter S, Gross P, Passauer J., 2004), pulmonary hypertension (Diller GP, van Eijl S, Okonko DO, Howard LS, Ali O, Thum T, Wort SJ, Bédard E, Gibbs JS, Bausachs J, Hobbs AJ, Wilkins MR, Gatzoulis MA, Wharton J., 2008) and Lupus (Westerweel PE, Luijten RK, Hoefer IE, Koomans HA, Derksen RH, Verhaar MC., 2007). In experimental studies after inducing AMI in animals, increased PBSC from GCSF injections contributed to a band of newly formed cardiac tissue which occupied more than 75% of the infarcted region and newly formed blood vessels supplied the infarcted tissue. By comparison the control animals were filled with scar tissue and no new blood vessels. In summary while only 17% of the untreated animals survived AMI, up to 73% of animals treated with GCSF survived with improved cardiac function and circulation (Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A,
Anversa P., 2001). Similar results have been shown in human studies (Wojakowski W, Tendera M, Zebzda A, Michalowska A, Majka M, Kucia M Maslankiewicz K, Wyderka R, Król M, Ochala A, Kozakiewicz K, Ratajczak MZ., 2006). This topic has been the subject of a recent meta-analysis (Abdel-Latif A, Bolli R, Zuba-Surma EK, Tleyjeh IM, Hornung CA, Dawn B., 2008). The outcome of stroke has been shown to recently correlate with the mobilized number of BMSC. In one study the magnitude of this mobilization was correlated with the patients functional recovery (Dunac A, Frelin C, Popolo-Blondeau M, Chatel M, Mahagne MH, Philip PJ., 2007). The ability of BMSCs to migrate and become insulin producing cells in the pancreas was shown by Ianus (Andreea Ianus, George G. Holz, Neil D. Theise, Mehboob, 2003). Hasagawa further demonstrated this is Recently the relationship linked the progression of diabetes to lower levels of PBSC. We will show with immunofluorescence the results of BMSC becoming insulin producing cells when analyzed 6 weeks post-transplantation of GFP-positive cells in irradiated mice. Mobilization of these cells is essential in streptozotocin induced diabetes which we also describe during our presentation (Hasagawa Y, Ogihara T, Yamada T, Ishigaki Y, Imai J, Uno K, Gao J, Kaneko K, Ishihara H, Sasano H, Nakachi H, Oka Y, Katagiri H., 2007).

Investigation of ECSV has been limited due to the significant risk of using G-CSF, the main stem cell mobilizer in clinical trials. Recently a new stem cell mobilizer (StemEnhance: SE) triggers a more gradual increase of PBSC and its safety allows for a sustained daily oral consumption over extensive periods of time. SE is an extract from the cyanophyta Aphanizomenon flos-aquae that concentrated a protein that was shown to be an L-selectin blocker. Oral consumption of 1 gram of SE have been shown to trigger an average 25% increase in the number of PBSC within 60 minutes. We plan on testing this natural agent in numerous disease states while at the same time using low level laser irradiation to potentiate its mobilization proliferation and differentation at its designated destination. We also will give anecdotes from our practice and present a paper showing SEs ability to allow recovery from injury of the anterior tibialis muscle in mice transplanted with GFP bone marrow stem cells after irradiation. SE enhanced significant recovery from the injury by mobilizing stem cells which was not seen in the contralateral tibialis muscle.

Bibliography:


In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion Andreea Ianus, George G. Holz, Neil D.


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