Influence of Low Level Laser Radiation on Migration of Stem Cells

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Abstract: The long term effects of low level laser therapy can involve treatment mechanisms connected with activation of stem cells.
In the current study migration of stem cells was tested under the influence of laser light alone as well as in case of combined influence of light and stromal cell-derived factor-1α (SDF-1α). This cytokine plays a role in lymphocyte trafficking, hematopoietic progenitor cell and stem cell homing.
To investigate the light influence on stem cells, we analyzed factor-dependent cell-Patersen (FDCP)-mix multipotent progenitor cells.
Migration of the stem cell line was tested using Transwell system (Corning, NY) under influence of red diode laser (λ=659.6 nm, 19.5 mW) or infrared diode laser (λ=958 nm, 36 mW) during 15 min at continuous wave, as well as in case of applying 150 ng/ml SDF-1α.
Group 1 cells were a group of control, group 2 cells received only red light irradiation, while group 3 cells had IR light irradiation. Group 4 cells were treated with 150 ng/ml SDF-1α. Group 5 cells were irradiated with red laser light in addition to 150 ng/ml SDF-1α, and group 6 cells by IR light and 150 ng/ml SDF-1α.
The count of migrated cells was 1496,5±409 (100%) in case of control. Red and IR laser light increased migration activity of stem cells up to 1892±283 (126%) and 2255,5±510 (151%) accordingly. Influence of SDF-1α was more significant, than effects of light irradiation alone 3365,5±489 (225%). Combined effects of light irradiation and SDF-1α were significantly stronger 5813±1199 (388%) for SDF-1α and red laser light, and 6391,5±540 (427%) for SDF-1α and IR laser light irradiation.
Preliminary study results showed that laser light irradiation can activate stem cell migration in vitro. The results are more reliable in the case of combined application of light and SDF-1α. These results are giving ground to consider that stem cell reactions to light irradiation can be one of the factors of light therapy.

Key words: low level laser irradiation, low level laser therapy, stem cells, SDF-1, stromal cell-derived factor-1

INTRODUCTION

More than 30 years ago first reports about biological effects of low doses of laser light were presented. Currently low level laser therapy (LLLT) is successfully applied in the treatment of numerous diseases and pathological conditions. LLLT exhibits positive effects for the treatment of disorders, having in common failure of blood supply with development of acute or chronic tissue hypoxia, different level of destruction of tissues, following decreased regenerative abilities of tissues and organs, defects in immune

system, and altered cell metabolism. At the same time some important mechanisms of influence of laser light on the body are still far to be fully understood [1 - 8].

Recent studies discovered important role of bone marrow hematopoetic stem cell (HSCs) for naturally occurred recovery and regeneration processes, following tissue hypoxia and injury. The three clinically important steps in this natural process are mobilization of stem cells from the bone marrow, homing of these cells to the site of injury, and differentiation of the stem cell into a functional cell of the injured tissue [9]. Different methods of stem cell therapy, the treatment method, based on mobilization and transplantation of stem cells, proves to be effective method of therapy for different disorders.

We proposed a hypothesis that wide range of positive effects following laser therapy can be connected to increased activity of stem cells in damaged tissues. To test that, we examined in vitro the influence of laser light on migration of stem cells in absence and in presence of stromal cell-derived factor-1 (SDF-1), a potent chemoattractor for lymphocytes, monocytes, HSCs, which plays a critical role in the stem cell migration towards areas of tissue injury and hypoxia.

MATERIALS AND METHODS

To investigate the light influence on stem cells, we analyzed factor-dependent cell-Patersen (FDCP)-mix multipotent progenitor cells. The FDCP-mix stem cell line was maintained in ISCOVE'S medium supplemented with 20% horse serum and penicillin/streptomycin in the presence of 20 ng/ml IL-3. The cells were supplied with fresh medium each 5 days. Migration of the stem cell line was tested using Transwell system (Corning, NY). The cells were washed with PBS once and re-suspended in the medium containing 0.1% BSA ($2x10^6$/ml). Then, 600 μl of the mixture was irradiated by red diode laser (λ=659.6 nm, 19.5 mW) or infrared diode laser (λ=958 nm, 36 mW) during 15 min at continuous wave. Next, 100 μl of the mixture ($2x10^5$ cells) was seeded into upper chambers of the Transwell system, and the filters were placed into the wells containing 600 μl of the medium with or without 150 ng/ml SDF-1α. The plate was incubated for 4 h (37°C, 5% CO_2, humidified atmosphere), after which the cells were collected and counted by a FACS sorter (Beckton Dickinson) during 1 min. All samples were performed in duplicate.

Group 1 cells are control group, group 2 cells received only red light irradiation, while group 3 cells – only IR light irradiation. Group 4 cells were treated with 150 ng/ml SDF-1α. Group 5 cells were irradiated with red laser light in addition to 150 ng/ml SDF-1α, and group 6 cells – IR light and 150 ng/ml SDF-1α.

RESULTS

Small amount of stem cells can migrate without SDF-1α or laser light influence. The count of migrated cells in control group was 1496,5±409 (Fig). This amount was considered as 100%. Red and IR laser light at the above mentioned dosage and methods of irradiation increased migration activity of stem cells up to 1892±283 (126%) and 2255,5±510 (151%) accordingly. Influence of SDF-1α was more noticeable, than effects of red or IR laser light irradiation alone - 3365,5±489 (225%). It

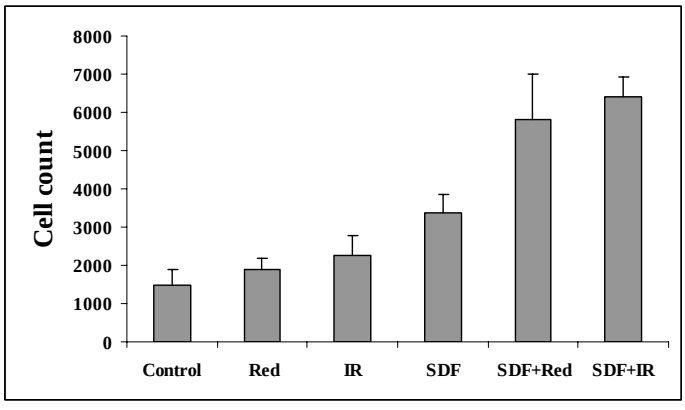

Fig. Influence of laser light and SDF-1α on stem cells migration.

is important to stress attention on the finding, that rate of stem cell migration towards the filter and SDF-1α containing medium was much higher after laser irradiation of cells - 5813±1199 (388%) for red laser light, and 6391,5±540 (427%) for IR laser light irradiation.

DISCUSSION

The main scientific result of this study is the fact, that red and infrared laser light irradiation can activate migration of stem cells in vitro. Moreover, red and IR laser radiation can up-regulate the rate of stem cell migration towards higher SDF-1α gradient.

How to explain the direct effects of mobility of stem cells in vitro under red and IR laser light irradiation, and use this fact for better understanding the wide range of therapeutic effects of laser therapy?

Modern medical science has accepted that every pathologic condition or disease should be treated according to its clinical stage and symptoms, considering its pathogenesis and etiology. Similar treatment methods can be applied only for the treatment of different diseases, having common pathogenesis.

Not very many examples of successful application of the similar or close therapy method for the treatment of different pathologies are known in modern medicine. Steroid hormone therapy is one of such cases.

Another illustration of successful application of the similar treatment techniques for treatment of different disorders is stem cell therapy, a novel treatment method, which is still under development. Growing data suggests, that transplanted stem cell can successfully and for long period of time improve heart myocardial contractility and other heart functions after myocardial infarction, can support neoangiogenesis in areas of tissue infarction and damage, can replace several cell types in tissues, including β-cells in diabetes models, neurons, cardiomyocytes, hematopoetic cells of different lineages and so on, as well as be useful in the treatment of atherosclerosis [9].

The main principle of stem cell therapy is the idea of replacement of damaged and dead cells in injured tissues and organs with new healthy ones. It is known, that severe stress, tissue hypoxia and damage mobilizes some hematopoetic stem cells (HSCs) from bone marrow to peripheral bloodstream. After that HSCs can migrate towards hypoxic tissues and reach them. Finally they can start to proliferate to the cells types, typical for that damaged tissues. HSCs in the tissues are also able to produce several cytokines, chemokines, growthfactors, improve survival of damaged cells and limit apoptosis. As a result of some tissue regeneration, improvement in the function of a damaged organ can be achieved. Similar and even stronger regeneration and treatment effects can be displayed after transplantation of fetal or adult HSCs to recipient [10-12].

Low laser light irradiation is one other example of application of the same factor for the treatment of number of disorders, which, at first glance, have nothing or very little in common in their pathogenesis. Laser light can accelerate wound and burn healing, improve condition of patients after myocardial infarction and stroke, can support hematopoiesis of bone marrow after X-ray radiation or during cancer chemotherapy, can help for the treatment of diabetic angiopathy and neuropathy, as well as reduce atherosclerotic plaque formation. In cellular and tissue level LLLT exhibits positive effects for the treatment of disorders, having in common failure of blood supply with development of acute or chronic tissue hypoxia, different level of destruction of tissues, following with decreased regenerative abilities of cells, as well as altered cell metabolism [6, 7, 13, 14].

One can see that the therapeutic applications of LLLT and stem cell therapy are very close. So, earlier we proposed the hypotheses that one of the mechanisms of light therapy includes acceleration of tissue repair due to better mobilization of stem cells to the spot

of injury after laser light irradiation [15]. That process should include several phases, including activation of stem cell migration towards area of tissue damage and hypoxia.

Stem cells are being investigated for their potential use in regenerative medicine. Stem cells share the following two defining characteristics: the capacity to differentiate into a spectrum of different cell types and the capacity to renew themselves [16]. The biological principle that underlies stem cell therapy is tissue-directed differentiation. For example, adult stem cells isolated from liver tissue and re-injected into liver become hepatocytes, whereas the same cells injected into myocardium become myocytes. [17] Stem cells have been engrafted into a broad spectrum of tissues, including regenerating bone, neural tissue, dystrophic skeletal muscle, and injured skeletal muscle. [18]. Myocardial regeneration is perhaps the most widely studied and debated example of stem cell plasticity. The most promising results have been obtained after transplantation and mobilization of bone marrow cells to the area of infarction.

The three clinically important steps in this natural process are mobilization of stem cells from the bone marrow, homing of these cells to the site of injury, and differentiation of the stem cell into a functional cell of the injured tissue [19].

Stem cell repair of cardiac and vascular tissue is a naturally occurring process after injury [20, 21] Circulating $CD34^+$ mononuclear cell counts and plasma levels of endothelial growth factor are significantly increased in patients with acute myocardial infarction, peaking on day 7 after onset [22]. Due to limitations of the naturally occurring repair process after myocardium infarction and other injuries or pathologies several stem cell transplantation strategies were proposed and tested.

At present, however, enthusiasm for the therapeutic potential of strategies of stem cell transplantation is limited by certain practical considerations. For example, the number of stem cells, required for injection for the treatment of myocardial infarction, can be harvested approximately from 6 l of donor blood [23].

Other important limitation for autologous bone marrow stem/progenitor cell mobilization is a recent finding, that circulating endothelial progenitor cells in patients with coronary heart disease are impaired with respect to number and functional activity. Moreover, Heeschen et al [24] reported that regeneration and functional ability of bone marrow-derived mononuclear cells (BM-MNCs) in patients with chronic ischemic cardiomyopathy (ICMP) are also limited. In spite of the fact that, the number of BM-MNCs isolated from bone marrow aspirates of 18 patients with ICMP and 8 healthy subjects s did not differ, the colony-forming capacity of BM-MNCs from patients with ICMP was significantly lower compared with BM-MNCs from healthy controls. Likewise, the migratory response to SDF-1 and vascular endothelial growth factor (VEGF) was significantly reduced in BM-MNCs derived from patients with ICMP compared with BM-MNCs from healthy controls. The reduced neovascularization capacity in vivo of BM-MNCs derived from patients with ICMP closely correlated with the in vitro assessment of SDF-1-induced migration and colony-forming capacity.

The need for development of new methods for mobilization, as well as for homing of stem cells to the site of injury is therefore evident.

Several growth factors, chemokines and cytokines are involved in the regulation of stem cell mobilization, homing and differentiation. Stromal cell-derived factor-1 (SDF-1) is one of them. SDF-1 is a chemokine playing an important role in the trafficking of hematopoietic stem cells. SDF-1 is expressed on stromal cells of various tissues. CXCR4 is the only known receptor for SDF-1 [25]. SDF-1/CXCR4 interaction is reported to play an important physiological role during embryogenesis in hematopoiesis, vascular development, cardiogenesis, and cerebellar development [26-28].

Recently, several investigators have reported that $CD34^+$ cells, classically considered to be hematopoietic stem cells, expressed CXCR4, and that SDF-1 could induce $CD34^+$ cell migration in vitro [29]. Accordingly, SDF-1 is considered as one of the key

regulators of hematopoietic stem cell trafficking between the peripheral circulation and bone marrow. SDF-1 has also been shown to effect $CD34^+$ cell proliferation and mobilization and to induce angiogenesis in vivo [30 -32].

Hattori et al [31] reported that plasma elevation of SDF-1 induced mobilization of mature and immature hematopoietic progenitors and stem cells, including endothelial progenitor cells (EPCs). However, application of granulocyte colony-stimulating factor (G-CSF) for stem cell mobilization is widely accepted nowadays.

Yamaguchi et al [23] studied the effects of SDF-1 on migration and accumulation of EPCs. SDF-1 induced EPCs migration in a dose dependent manner in vitro. The magnitude of migration was similar to that induced by VEGF. Authors also reported that locally (in hind-limb ischemic muscle of experimental animals) administered SDF-1 could augment the local accumulation of transplanted EPCs from peripheral blood, thereby resulting in enhanced neovascularization. As a result, cell transplantation not only improved neovascularization but also reduced adverse biological consequences such as limb necrosis and auto-amputation in the mouse ischemic hind-limb model. These studies also disclosed that systemic EPCs transplantation improved myocardial neovascularization and cardiac function corresponding to reduced left ventricular scarring. Authors concluded that, at least under the experimental conditions used in the study, the effect of SDF-1 on neovascularization appears to result primarily from its ability to enhance the recruitment and incorporation of transplanted EPCs.

Damas at al. [33] reported that SDF-1α, at least in high concentrations, may mediate anti-inflammatory and matrix-stabilizing effects in unstable angina. These effects may promote plaque stabilization, and therapeutic intervention that enhances SDF-1 α activity could potentially be beneficial in acute coronary syndromes. Authors demonstrated significantly altered SDF-1/CXCR4 expression in patients with angina, with particularly marked changes in those with unstable disease, with low SDF-1 levels in plasma and altered expression of its corresponding receptor on peripheral blood mononuclear cells (PBMC). In contrast to the raised plasma levels of inflammatory chemokines in patients with angina plasma levels of SDF-1 and the surface expression of its corresponding receptor (CXCR4) on PBMC appear to be down-regulated in these patients. Thus, although persistent inflammation may involve up-regulation of inflammatory chemokines, recent studies suggest that inflammatory cytokines (eg, TNF-α and IL-1) may decrease the expression of SDF-1 and CXCR4.

Future progress of stem therapy techniques probably will include development of incubation methods for enhancement stem cell mobility and homing ability, as well as for faster proliferation into desire tissue cells. Increasing migration abilities will help to achieve better and faster results.

The ability of laser light to activate migration and mobility of different cells is well known. It was noticed, that irradiation of sperm cells in vitro can increase their mobility and fertility [34]. Moreover, this effect is more pronounced in case of damaged cells with low mobility rate. This gives a ground to assume that laser light irradiation in certain dosage and condition can improve functional abilities of cells. Future experiments are required to ascertain if stem cells respond to the laser light the same way.

The main finding on this study is that red and IR laser light can stimulate stem cell migration in vitro, and especially increase migration towards SDF-1α gradient. Stem cell ability to migrate towards tissues with higher SDF-1 concentration is one of the key mechanisms of stem cell homing. These results are giving ground to speculate that activation of stem cell migration can be one of the mechanisms of low level laser therapy. Taking into consideration that the combined of SDF-1 and laser irradiation had the strongest effect on stem cell homing, it would be reasonable to assume that this combination could be used in not only increasing the activity of stem cells but also in determining the main area of stem cell mobilization and homing. The current study did

not aim to study the mechanisms of increased migration ability, which will be study in the future. But it is possible to suggest following explanation: laser irradiation can change the metabolism of stem cells, increase ATP production and so increase the migration, as well as up-regulate CXCR4 receptor expression or syntheses de novo. More studies are required to test if the laser light irradiation in vivo is able to make homing of transplanted stem cells to the area of damage more efficient, to check the influence of laser light on the mobilization rate of stem cells from bone marrow, to investigate if laser light can enhance functional abilities of stem cells. These studies would be desirable for better understanding of the mechanisms of laser therapy and for development of more effective methods of stem cell therapy.

References

1. Tuner J. and Hode L. Low Level Laser Therapy: Clinical Practice and Scientific Background, Prima Books, Grängesberg, Sweden, 1999.
2. Karu T. The Science of Low Power Laser Therapy, Gordon & Breach, London, 1998.
3. Baxter G.D. Therapeutic Lasers: Theory and Practice, Churchill Livingstone, London, 1994.
4. Simunovic Z., Ed. Lasers in Medicine and Dentistry, Vitgraf, Rijeka (Croatia), 2000.
5. Zhukov B.N. and Lysov N.A. Laser irradiation in experimental and clinical angiology (in Russian), Samara (Russia), 1996.
6. Kozlov V.I., et al. Bases of laser physio- and reflexo-therapy (in Russian), Zdorovje, Samara (Russia), 1993.
7. Paleev N.R. Ed. Phototherapy (in Russian), Meditsina, Moscow (Russia), 2001.
8. Skobelkin O. K. Ed. Application of low-intensive lasers in clinical practice (in Russian). Moscow, 1997.
9. Forrester J, Price M, Makkar R. Stem Cell Repair of Infarcted Myocardium. An Overview for Clinicians. Circulation. 2003;108:1139–1145.
10. Orlic D., Hill J., Arai A. Stem Cells for Myocardial Regeneration Circulation Research. 2002;91:1092.
11. Hodgson D., Behfar A., Zingman L.V., Kane G.C., Perez-Terzic C., Alekseev A.E., Puceat M., and Terzic A. Stable benefit of embryonic stem cell therapy in myocardial infarction. Am J Physiol Heart Circ Physiol, August 1, 2004; 287(2): H471 - H479.
12. Ozbaran M., Omay S. B., Nalbantgil S., Kultursay H., Kumanlioglu K., Nart D., and Pektok E. Autologous peripheral stem cell transplantation in patients with congestive heart failure due to ischemic heart disease. Eur. J. Cardiothorac. Surg., March 1, 2004; 25(3): 342 - 350.
13. Brill A.G., Shenkman B., Brill G.E. et al. Blood irradiation by He-Ne laser induces a decrease in platelet responses to physiological agonists and an increase in platelet cyclic GMP. Platelets. 2000. Vol. 11. P. 87-93.
14. Mester A. Biostimulative effect in wound healing by continuous wave 820 nm laser diode. Lasers in Med Science, abstract issue July 1988, No. 289.
15. Gasparyan L.V. Stem cells and therapeutic effect of light irradiation (in Russian). Collection of abstracts of the 10th International Conference of Quantum Medicine, Moscow, 2003, pp. 43-44.
16. Graf T. Differentiation plasticity of hematopoietic cells. Blood. 2002;99:3089–3101.
17. Malouf NN, Coleman WB, Girsham JW, et al. Adult-derived stem cells from the liver become myocytes in the heart in vivo. Am J Pathol. 2001;158:1929–1935.

18. Donovan PJ, Gearhart J. The end of the beginning for pluripotent stem cells. Nature. 2001;414:92–97.
19. Forrester J, Price M, Makkar R. Stem Cell Repair of Infarcted Myocardium. An Overview for Clinicians. Circulation. 2003;108:1139–1145.
20. Beltrami AP, Urbanek K, Kajstura J, et al. Evidence that human cardiac myocytes divide after myocardial infarction. N Engl J Med. 2001;344:1750–1757.
21. Gill M, Dias S, Hattori K, et al. Vascular trauma induces rapid but transient mobilization of VEGFR2(+)AC133(+) endothelial precursor cells. Circ Res. 2001;88:167–174.
22. Shintani S, Murohara T, Ikeda H, et al. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation. 2001;103:2776–2779.
23. Yamaguchi J, Kusano K, Masuo O, at al. Stromal Cell–Derived Factor-1 Effects on Ex Vivo Expanded Endothelial Progenitor Cell Recruitment for Ischemic Neovascularization. Circulation. 2003;107: 1322–1328.
24. Heeschen C, Lehmann R, Honold J, Assmus B, Aicher A, Walter DH, Martin H, Zeiher AM, Dimmeler S. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation. 2004;109(13):1615-22.
25. Bleul CC, Farzan M, Choe H, et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature. 1996;382:829–833.
26. Nagasawa T, Hirota S, Tachibana K, et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature. 1996;382:635–638.
27. Tachibana K, Hirota S, Iizasa H, et al. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature. 1998; 393:591–594.
28. Zou YR, Kottmann AH, Kuroda M, et al. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature. 1998;393:595–599.
29. Mohle R, Bautz F, Rafii S, et al. The chemokine receptor CXCR-4 is expressed on $CD34^+$ hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. Blood. 1998;91:4523–4530.
30. Lataillade JJ, Clay D, Dupuy C, et al. Chemokine SDF-1 enhances circulating $CD34^+$ cell proliferation in synergy with cytokines: possible role in progenitor survival. Blood. 2000;95:756–768.
31. Hattori K, Heissig B, Tashiro K, et al. Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells. Blood. 2001;97:3354–3360.
32. Salcedo R, Wasserman K, Young HA, et al. Vascular endothelial growth factor and basic fibroblast growth factor induce expression of CXCR4 on human endothelial cells: in vivo neovascularization induced by stromal derived factor-1α. Am J Pathol. 1999;154:1125–1135.
33. Damas J, Wæhre T, Yndestad A, et al. Stromal Cell–Derived Factor-1a in Unstable Angina. Circulation. 2002;106:36-42.
34. Pyrikova S.I et al. Effect of laser exposure on human seminal fluid (in Russian). Clinical and laboratory diagnosis. 1998;5:15-16.