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Can laser therapy modify the secretion of the tissue-type plasminogen activator and its inhibitor in an endothelial cell culture under hyperglycemia?

Krzysztof Góralczyk[1, A], Justyna Szymańska[2, B], Zbigniew Ziętek[3, C] ✉, Katarzyna Szot[1], Jacek Fisz[2, D], Danuta Rość[1, E]

[1] Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Department of Pathophysiology, Curie-Skłodowskiej 9, 85-094 Bydgoszcz, Poland
[2] Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Department of Physiotherapy, Techników 3, 85-801 Bydgoszcz, Poland
[3] Pomeranian Medical University in Szczecin, Department of Normal and Clinical Anatomy, Powstańców Wlkp. 72, 70-111 Szczecin, Poland

[A] ORCID: 0000-0002-0804-6412; [B] ORCID: 0000-0003-4091-3704; [C] ORCID: 0000-0003-4049-7851; [D] ORCID: 0000-0003-1128-5286; [E] ORCID: 0000-0002-2971-9882

✉ zzietek@poczta.onet.pl

ABSTRACT

Introduction: The effect of low-level laser therapy on the secretion of tissue-type plasminogen activator (t-PA) and its inhibitor (PAI-1) in an endothelial cell (EC) culture under hyperglycemia is the subject of the presented work.

Hyperglycemia associated with diabetes causes vascular EC dysfunction. Low-level laser therapy is a good method to support the pharmacological treatment of diabetes complications.

Materials and methods: We used lasers of 2 wavelengths: 635 nm and 830 nm with dose of 2 J/cm2. The experiment was performed *in vitro* in 4 groups of EC: 1 – no glucose in culture medium, no irradiation (control), 2 – glucose, no irradiation, 3 – glucose, laser 635 nm, 4 – glucose, laser 830 nm. After 2 irradiations, cells were counted and the t-PA and PAI-1 antigen (Ag) concentration in the supernatant was measured.

Results: In group 2, we observed a statistically significant lower number of cells ($p < 0.0001$) and a higher concentration of t-PA:Ag and PAI-1:Ag ($p < 0.05$) compared to the control group. However, in groups 3 and 4, the number of cells increased and the concentration of t-PA:Ag and PAI-1:Ag decreased compared to group 2 and nearly reached the values in the control group.

Conclusions: Hyperglycemia affects the fibrinolytic activity of ECs which is manifested by a significant increase in t-PA:Ag and PAI:Ag concentrations – recognized markers of endothelial damage. Irradiation of ECs by a low-power laser caused attenuation of the adverse effects of hyperglycemia. A tendency towards a decrease in t-PA:Ag and PAI-1:Ag concentration in the supernatant was observed with a significant increase in the number of cells to values close to control.

Keywords: low-level laser therapy; endothelial cell culture; t-PA; PAI-1; hyperglycemia.

INTRODUCTION

A high glucose level in diabetes mellitus (DM) causes a dysfunction in the vascular endothelium that lines the interior surface of blood vessels. The endothelium plays a key role in maintaining vascular homeostasis via the secretion of active agents involved in many biological processes such as hemostasis, angiogenesis and inflammation [1]. Hyperglycemia induces changes such as an increase of plasminogen activator inhibitor-1 (PAI-1) and endothelin-1 or a decrease in nitric oxide (NO) in the fibrinolytic and vasoactive components. Nitric oxide, by dilating vessels and inhibiting thrombocyte adhesion, is an important factor preventing thrombosis [2, 3]. Under conditions of elevated glucose levels, the weakening of endothelial fibrinolytic activity is also associated with an impairment in the binding of tissue-type plasminogen activator (t-PA) with inactive proenzyme plasminogen, which in turn leads to a decrease in plasmin generation – an active enzyme that is responsible for thrombus degradation [4]. A balance in t-PA and its inhibitor PAI-1 is a requirement for the proper functioning of the hemostatic system. Impaired fibrinolysis under hyperglycemic conditions disturbs this state leading to pro-thrombotic complications [5]. Elevated levels of t-PA and PAI-1 were observed in diabetic patients with cardiovascular complications and in patients with ischemic heart disease [6, 7]. Plasminogen activator inhibitor-1 is an independent risk factor for the development of diabetes in healthy people as elevated levels were observed in patients with DM and cardiovascular diseases [8]. High levels of t-PA and PAI-1 are considered a predictor of vascular complications [9, 10]. Many studies have shown that hyperglycemia reduces cell proliferation and migration, increases apoptosis and also affects the rate of cell aging [11, 12, 13, 14] which interferes with the wound healing process.

The treatment of diabetes and its vascular complications, such as leg ulcers, are still a huge challenge for modern medicine. A good method for supporting the pharmacological treatment of diabetes complications is low-level laser therapy (LLLT) which was developed in the 1960s. During irradiation, photons are absorbed in the mitochondria of the cells, allowing for an improvement in energy metabolism and stabilization of the cell membrane [15, 16]. Low-level laser therapy has a biostimulative effect without a temperature increase, is painless, non-invasive and has no side effects, brings pain relief and affects faster recovery [17].

This work is a continuation of our *in vitro* studies [18] on the effect of hyperglycemia on the vascular endothelial cells (ECs) of the human umbilical vein endothelial cells (HUVECs) line. The aim of the current pioneering research was to evaluate the effect of a low-level laser at 2 wavelengths of 635 nm (visible light) and 830 nm (infrared) on the t-PA and PAI-1 antigen (Ag) concentration in the HUVEC culture supernatant in hyperglycemia.

MATERIALS AND METHODS

Human umbilical vein endothelial cell isolation and culture

Human umbilical vein ECs were isolated and cultured under hyperglycemic conditions as described previously [18]. The main part of the experiment was performed on cells cultured in 6-well plates (seeding density of 7.5 x 10^3 cells/cm^2) and derived from 3 independent isolations.

Characteristics of laser irradiation and the measurement of analyzed parameters

In the experiment we used a semiconductor-based laser in a set for the biological structure irradiation, created by colleagues from the Bialystok University of Technology [19]. Laser irradiation with wavelengths of 635 nm (power density 1.875 mW/cm^2) and 830 nm (power density 3.75 mW/cm^2) were applied. The exposure dose was 2 J/cm^2. The irradiation conditions in the study group were identical to those described in our previous experiment [18]. The cells from the 1st group (control) were not exposed to laser irradiation and the culture medium did not contain glucose. The 2nd group of cells was not irradiated either but the medium contained glucose at a level of 30 mM/L. The 3rd and 4th group were cultured in a medium with 30 mM/L glucose and irradiated with wavelengths of 635 nm and 830 nm, respectively.

The levels of t-PA antigen and PAI-1 antigen were determined in the supernatant after 7 days using the ELISA test (eBioscience, Vienna, Austria) according to the manufacturer's instructions. The cells were counted in all wells of the experimental plates by a Burker hemocytometer using trypan blue dye according to Basso et al. [20].

Statistical analysis

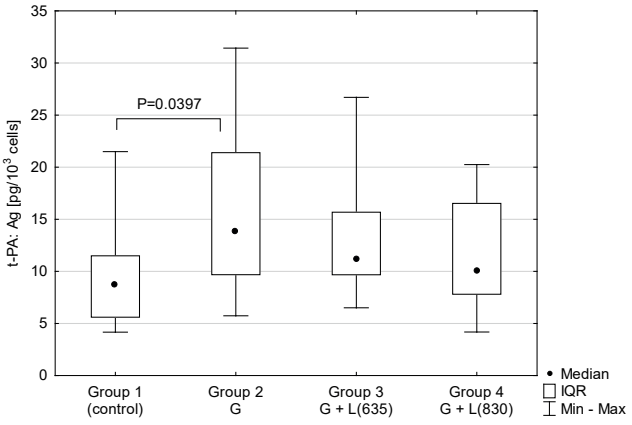
Statistical analysis was performed using Statistica 13.1 (Dell Inc. Tulsa, USA). The one-way ANOVA with *post hoc* test was used. The Fisher test was used for normal distribution variables (cell numbers) and the Kruskal–Wallis test was used for non-normal distribution variables: tissue-type plasminogen activator antigen (t-PA:Ag) and plasminogen activator inhibitor-1 antigen (PAI-1:Ag). Statistical significance was defined as $p < 0.05$. Only statistically significant values were given in the table and figures. The mean ±standard deviation or median and interquartile range was used for the statistical description.

Ethical considerations

The approval of the Bioethics Commission of the NCU Collegium Medicum in Bydgoszcz was obtained, No KB/135/2009 with the last annex of 19.06.2018.

RESULTS

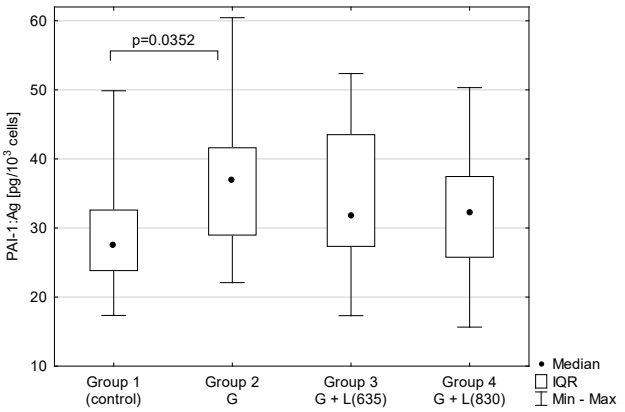
Figure 1 shows the results regarding the effect of low-level laser irradiation on t-PA:Ag concentration in the supernatant of the HUVEC culture. This was cultured with an elevated glucose level in the medium (glucose concentration 30 mM/L). The level of t-PA:Ag in the control group (group 1) without glucose in the medium and not irradiated by the laser was 8.69 pg/10^3 cells. In group 2, which contained glucose in the medium and was not irradiated, the concentration of t-PA:Ag was significantly higher (13.86 pg/10^3 cells) in comparison to the control group ($p = 0.0397$). In groups 3 and 4, cells were growing in the medium with glucose and irradiated by the laser at a wavelength of 635 nm and 830 nm, respectively. The concentration of t-PA:Ag in group 3 was slightly lower in comparison with group 2, and in group 4 was notably lower. This value amounts to 10.09 pg/10^3 cells which is a 37% decrease in relation to group 2. However, these differences were not statistically significant.



G – glucose in culture medium; L(635) – laser irradiation with a wavelength of 635 nm; L(830) – laser irradiation with a wavelength of 830 nm; IQR – interquartile range; Min – minimum; Max – maximum

FIGURE 1. The antigen concentration of tissue-type plasminogen activator in the supernatant of human umbilical vein endothelial cells line endothelial cell culture depending on hyperglycemia and laser irradiation of different wavelengths

Figure 2 shows the concentration of PAI-1:Ag in analogous groups as in Figure 1. The concentration of PAI-1:Ag in the control group was 27.53 pg/10^3 cells and was 34% lower than in group 2 ($p = 0.0352$). Laser irradiation at a wavelength of 635 nm in a hyperglycemic environment (group 3) reduced the PAI-1:Ag concentration to 31.72 pg/10^3 cells and a similar effect was obtained for the laser at a wavelength of 830 nm (group 4). These values approximated to values in the control group, however, differences in relation to group 2 were not statistically significant. There were also no differences in the effects of the 2 wavelengths.



G – glucose in culture medium; L(635) – laser irradiation with a wavelength of 635 nm; L(830) – laser irradiation with a wavelength of 830 nm; IQR – interquartile range; Min – minimum; Max – maximum

FIGURE 2. The antigen concentration of plasminogen activator inhibitor-1 in the supernatant of human umbilical vein endothelial cells line endothelial cell culture depending on hyperglycemia and laser irradiation of different wavelengths

Table 1 shows the number of HUVECs was highest in the control group and the lowest number of cells was observed in group 2. The difference was statistically significant ($p < 0.0001$) in comparison with the control group. In group 3, the number of cells was slightly higher compared to group 2 and in group 4 it reached a level closer to the control group. The differences between groups 3–4 were statistically insignificant.

TABLE 1. The number of human umbilical vein endothelial cells (N*105) depending on hyperglycemia and laser irradiation of different wavelengths

Control	Glucose	Glucose + Laser 635 nm	Glucose + Laser 830 nm	p
Group 1	Group 2	Group 3	Group 4	
6.82 ±0.96	4.87 ±0.77	5.35 ±0.82	6.11 ±0.71	<0.0001[1 vs. 2] <0.0001[1 vs. 3] <0.0004[2 vs. 4]

The values were described as M ±SD; p – statistical significance according to one-way ANOVA with *post hoc* test.

DISCUSSION

Tissue-type plasminogen activator is the major physiological plasminogen activator. Its concentration depends on the concentration of its inhibitor PAI-1. A significant portion of t-PA circulates in plasma in complexes with PAI-1. The proteins of the fibrinolytic system are also involved in tissue remodeling, angiogenesis and wound healing. Synthesis of t-PA and PAI-1 takes place primarily in the ECs of blood vessels and in the liver and vascular smooth muscle cells [21, 22]. This is why our *in vitro* experimental model is based on ECs of the HUVEC line grown in a medium containing glucose at a concentration imitating the conditions found in the blood of patients with diabetes (30 mM/L). This level of glucose concentration in the culture medium is commonly used in this type of *in vitro* study. An elevated level of blood glucose promotes non-enzymatic glycation of proteins (including activators and inhibitors of the hemostatic system) and contributes to the release of advanced glycation end products and oxidative stress [23]. Adverse processes then occur in the mitochondrial respiratory chain and there is an increase in the production of free radicals and peroxide anions [1]. Advanced glycation end products bind to specific receptors and activate cells through the proinflammatory nuclear factor (NF-κB) which increases the gene expression of proinflammatory cytokine, growth and hemostasis factors [24].

The results of our experiment confirm the adverse effect of hyperglycemia on vascular ECs. It caused a decrease in ECs numbers in comparison to the control (Tab. 1) as well as a simultaneous increase in the concentration of t-PA:Ag and PAI-1:Ag in the supernatant (Fig. 1, 2). The difference between these values was over 30% and statistically significant. Some researchers have observed a decrease in t-PA:Ag levels in patients with diabetes [3], but our research shows that hyperglycemia causes damage to ECs (and a reduction in their numbers, Tab. 1) and at the same time, induces an increased concentration of t-PA:Ag and PAI-1:Ag in the supernatant (Fig. 1, 2). as is confirmed by other researchers [2, 6]. This can be explained by a simultaneous increase in the formation of t-PA-PAI-1 complexes under conditions of high PAI-1:Ag concentration. The research of Pandolfi et al. indicates that hyperglycemia causes an increase in t-PA:Ag levels, but its active form is radically reduced because t-PA:Ag in the complex with PAI-1:Ag is fibrinolytic inactive [25]. Knudsen et al. show that abnormal glucose regulation strengthens fibrinolytic stress and is associated with high levels of t-PA:Ag and PAI-1:Ag in patients after myocardial infarction [8]. For a complete understanding of these processes, further research should be complemented by PAI-1 activity results. The obtained results also suggest the necessity of undertaking immunohistochemistry research on ECs cultures to obtain knowledge about the location of the studied proteins and damage caused by hyperglycemia.

The number of circulating endothelial progenitor cells is significantly reduced in diabetes, as is their proliferation, adhesion and migration [26]. In hyperglycemia, there is also an impairment in angiogenesis and neovascularization. It also induces aging in the vascular cells of mice, which is manifested by an increase in PAI-1, p53 and p21 [27]. This is confirmed by *in vitro* studies on HUVEC. Along with an increase in the number of passages, they proliferate more slowly and show increased expression of PAI-1 [12]. In our study, the number of HUVECs was significantly lower (by 29%) under hyperglycemic conditions when compared to the control group (normal glucose concentration) – Table 1. Considering the high concentration of t-PA:Ag and PAI-1:Ag in the supernatant from the EC culture with glucose (Fig. 1, 2), it is possible to assume that hyperglycemia has led to some of these cells disintegrating and releasing t-PA and PAI-1.

Nowadays, standard treatment methods for diabetic complications such as ulcers and hard-to-heal wounds are often supported by unconventional treatments. Phototherapy using low-power lasers or light-emitting diodes have promising effects. Low-level laser therapy stimulates microcirculation and angiogenesis which accelerates the healing of wounds [28].

In a study conducted by Feitosa et al. on DM patients, the use of a 30 mW laser with a wavelength of 632.8 mm at a dose of 4 J/cm^2 resulted in a significant reduction in wound size compared to the control group [29]. The mechanism of action of LLLT comprises an absorption of photons by irradiated cells.

The main role in the mitochondrial respiratory chain is played by cytochrome C oxidase. Its concentration increases under the influence of low-level laser irradiation. This enzyme catalyzes the reduction of oxygen for energy metabolism. It increases the transport of electrons in the respiratory chain and increases oxygen consumption within the mitochondria [16]. This process is accompanied by the synthesis of adenosine. Various transcription processes are initiated as well as the activation of signaling pathways [15, 30]. As a consequence, this has an impact on the treatment improvement due to the release of NO and suppression of reactive oxygen species and NF-κβ. Low-level laser therapy also improves cell viability and proliferation which are attenuated by hyperglycemia [11, 13, 31]. In our research, the beneficial effect of laser therapy is manifested by a decrease in the concentration of t-PA:Ag and PAI-1:Ag in the groups of irradiated cells compared to non-irradiated cells growing in the medium containing an elevated glucose level (Fig. 1, 2). In addition, the number of cells growing in hyperglycemic conditions in the groups irradiated by lasers (3–4) was higher than in the non-irradiated group (group 2). A difference of 25% was observed between groups 2 and 4 ($p = 0.0004$) – Table 1. Differences between the 2 waves are not statistically significant, although slightly more favourable effects were noted for the 830 nm wave (Fig. 1, 2). The research of Houreld and Abrahamse on diabetic-wounded fibroblast cells indicates better cell proliferation under the influence of a 632.8 nm wave than 830 nm [32]. Numerous studies confirm differences in the effects of LLLT at different wavelengths but this mechanism has not yet been explained. This difference may be caused by the activation of ion channels by certain wavelengths. Furthermore, differences in the optimal dose of radiation (J/cm^2) for particular wavelengths can also have an impact [33].

CONCLUSIONS

Hyperglycemia caused an increase in the concentration of t-PA:Ag and PAI-1:Ag in the supernatant of an EC culture with a simultaneous decrease in the number of cells, indicating that some of them had been damaged or were apoptotic.

Irradiation of ECs by a low-power laser caused an attenuation of the adverse effects of hyperglycemia. A tendency towards a decrease in the concentration of t-PA:Ag and PAI-1:Ag in the supernatant was observed with a significant increase in the number of cells to values close to the control. There is a need to continue research in this area.

REFERENCES

1. van den Oever IAM, Raterman HG, Nurmohamed MT, Simsek S. Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. Mediators Inflamm 2010;2010:792393. doi:10.1155/2010/792393.
2. Donnini D, Del Terra E, Ambesi-Impiombato FS, Curcio F. New *in vitro* model to study high glucose-dependent endothelial dysfunctions. Biochimie 2003;85(7):701-5.
3. Tjärnlund-Wolf A, Brogren H, Lo EH, Wang X. Plasminogen activator inhibitor-1 and thrombotic cerebrovascular diseases. Stroke 2012;43(10):2833-9.
4. Dai H, Yu Z, Fan X, Liu N, Yan M, Chen Z, et al. Dysfunction of annexin A2 contributes to hyperglycaemia-induced loss of human endothelial cell surface fibrinolytic activity. Thromb Haemost 2013;109(6):1070-8.
5. Lemkes BA, Hermanides J, Devries JH, Holleman F, Meijers JCM, Hoekstra JBL. Hyperglycaemia: a prothrombotic factor? J Thromb Haemost 2010;8(8):1663-9.
6. Galajada P, Martinka E, Kubisz P, Stasko J, Mokan M. Tissue plasminogen activator and diabetes mellitus. Vnitr Lek 1998;44(11):661-4.
7. Geppert A, Graft S, Beckmann R, Hornykewycz S, Schuster E, Binder BR, et al. Concentration of endogenous t-PA antigen in coronary artery disease: relation to thrombotic events, aspirin treatment, hyperlipidemia, and multivessel disease. Arterioscler Thromb Vasc Biol 1998;18(10):1634-42.
8. Knudsen EC, Seljeflot I, Abdelnoor M, Eritsland J, Mangschau A, Müller C, et al. Elevated levels of PAI-1 activity and t-PA antigen are associated with newly diagnosed abnormal glucose regulation in patients with ST-elevation myocardial infarction. J Thromb Haemost 2011;9(8):1468-74.
9. Thögersen AM, Jansson JH, Boman K, Nilsson TK, Weinehall L, Huhtasaari F, et al. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the fibrinolytic system as an independent primary risk factor. Circulation 1998;98(21):2241-7.
10. Harte AL, McTernan PG, McTernan CL, Smith SA, Barnett AH, Kumar S. Rosiglitazone inhibits the insulin-mediated increase in PAI-1 secretion in human abdominal subcutaneous adipocytes. Diabetes Obes Metab 2003;5(5):302-10.
11. Gao X, Xing D. Molecular mechanisms of cell proliferation induced by low power laser irradiation. J Biomed Sci 2009;16(1):4. doi:10.1186/1423-0127-16-4.
12. Yanaka M, Honma T, Sato K, Shinohara N, Ito J, Tanaka Y, et al. Increased monocytic adhesion by senescence in human umbilical vein endothelial cells. Biosci Biotechnol Biochem 2011;75(6):1098-103.
13. Hendudari F, Piryaei A, Hassani SN, Darbandi H, Bayat M. Combined effects of low-level laser therapy and human bone marrow mesenchymal stem cell conditioned medium on viability of human dermal fibroblasts cultured in a high-glucose medium. Lasers Med Sci 2016;31(4):749-57.
14. Vaughan DE, Rai R, Khan SS, Eren M, Ghosh AK. Plasminogen activator inhibitor-1 is a marker and a mediator of senescence. Arterioscler Thromb Vasc Biol 2017;37(8):1446-52.
15. Karu T. Mitochondrial mechanisms of photobiomodulation in context of new data about multiple roles of ATP. Photomed Laser Surg 2010;28(2):159-60.
16. Wang X, Tian F, Soni SS, Gonzales-Lima F, Liu H. Interplay between up-regulation of cytochrome-c-oxidase and hemoglobin oxygenation induced by near-infrared laser. Sci Rep 2016;6:30540.
17. Kajagar BM, Godhi AS, Pandit A, Khatri S. Efficacy of low level laser therapy on wound healing in patients with chronic diabetic foot ulcers – a randomised control trial. Indian J Surg 2012;74(5):359-63.
18. Góralczyk K, Szymańska J, Szot K, Fisz J, Rość D. Low-level laser irradiation effect on endothelial cells under conditions of hyperglycemia. Lasers Med Sci 2016;31(5):825-31.
19. Gryko Ł, Gilewski M, Szymańska J, Zając A, Rość D. The concept of the set to objectification of LLLT exposure. Proceedings of SPIE: Laser Technology 2012. App Lasers 2013;8703:870302.
20. Basso FG, Oliveira CF, Kurachi C, Hebling J, de Souza Costa CA. Biostimulatory effect of low-level laser therapy on keratinocytes *in vitro*. Lasers Med Sci 2013;28(2):367-74.
21. Collen D, Lijnen HR. Tissue-type plasminogen activator: a historical perspective and personal account. J Thromb Haemost 2004;2(4):541-6.
22. Gils A, Declerck PJ. Plasminogen activator inhibitor-1. Curr Med Chem 2004;11(17):2323-34.

23. Yamagishi S, Nakamura K, Matsui T, Ueda S, Noda Y, Imaizumi T. Inhibitors of advanced glycation end products (AGEs): potential utility for the treatment of cardiovascular disease. Cardiovasc Ther 2008;26(1):50-8.
24. Goldberg RB. Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. J Clin Endocrinol Metab 2009;94(9):3171-82.
25. Pandolfi A, Iacoviello L, Capani F, Vitacolonna E, Donati MB, Consoli A. Glucose and insulin independently reduce the fibrinolytic potential of human vascular smooth muscle cells in culture. Diabetologia 1996;39(12):1425-31.
26. Zhang J, Zhang X, Li H, Cui X, Guan X, Tang K, et al. Hyperglycaemia exerts deleterious effects on late endothelial progenitor cell secretion actions. Diab Vasc Dis Res 2013;10(1):49-56.
27. Chen H, Wan Y, Zhou S, Lu Y, Zhang Z, Zhang R, et al. Endothelium-specific SIRT1 overexpression inhibits hyperglycemia-induced upregulation of vascular cell senescence. Sci China Life Sci 2012;55(6):467-73.
28. Rocha Jr AM, Vieira BJ, de Andrade LC, Aarestrup FM. Effects of low-level laser therapy on the progress of wound healing in humans: the contribution of *in vitro* and *in vivo* experimental studies. J Vasc Bras 2007;6(3):258-66.
29. Feitosa MCP, Carvalho AFM, Feitosa VC, Coelho IM, Oliveira RA, Arisawa EAL. Effects of the low-level laser therapy (LLLT) in the process of healing diabetic foot ulcers. Acta Cir Bras 2015;30(12):852-7.
30. Silveira PC, da Silva LA, Pinho CA, De Souza PS, Ronsani MM, da Luz Scheffer D, et al. Effects of low-level laser therapy (GaAs) in an animal model of muscular damage induced by trauma. Lasers Med Sci 2013;28(2):431-6.
31. Ayuk SM, Houreld NN, Abrahamse H. Effect of 660 nm visible red light on cell proliferation and viability in diabetic models *in vitro* under stressed conditions. Lasers Med Sci 2018;33(5):1085-93. doi: 10.1007/s10103-017-2432-2.
32. Houreld N, Abrahamse H. Laser light influences cellular viability and proliferation in diabetic-wounded fibroblast cells in a dose- and wavelength-dependent manner. Lasers Med Sci 2008;23(1):11-8.
33. Wang Y, Huang YY, Wang Y, Lyu P, Hamblin MR. Photobiomodulation of human adipose-derived stem cells using 810nm and 980nm lasers operates via different mechanisms of action. Biochim Biophys Acta Gen Subj 2017;1861(2):441-9.