The effects of red/infrared light treatment on the total protein concentration in lymphocytes

Amela Softic[1,*], *Nadira Ibrisimovic Mehmedinovic*[1], and *Aldina Kesic*[1]

[1]Faculty of natural sciences, University of Tuzla, Urfeta Vejzagića 4, Tuzla, Bosnia and Herzegovina

Abstract. The effectiveness of red light therapy (RLT) on cellular activity is important in determining if its usage is suitable for the treatment of various health conditions. In order to better understand the molecular and metabolic mechanisms related to RLT, our preliminary study of the effect of LED light sources 660 nm and 850 nm on the proliferation of human lymphocyte cells was further extended to analyze its effect on the synthesis of cellular enzymes. Using the Bradford method, the total concentration of proteins in lymphocytes was monitored for different time parameters and conditions of exposure and in relation to the untreated (non-irradiated) control cells. Our results show a higher protein concentration for all irradiated samples when compared to the unirradiated samples and it was especially increased for multiple exposures to red/infrared light. Since the used method cannot answer whether the increased protein concentration has positive or negative connotations, it is important to continue the research and include additional methods that test oxidative stress and the structure of chromosomes on a larger number of samples in order to gain additional understanding of the beneficial impact of RLT on lymphocyte cells.

Keywords: RLT, lymphocytes, protein concentrations

1 Background

Red light therapy (RLT), also known as low level laser therapy (LLLT) is a light-based treatment that uses red or near-infrared wavelengths to stimulate cellular metabolism and gene/protein expression affecting healing and functional regulation. It is in use for more than 50 years and has been progressively applied in medicine, specifecally physiotherapy and cell biology. In has become widely known as photobiomodulation (PMB) to better describe its non-thermal biological mechanisms. Investigation of how red light therapy affects lymphocytes is of significance in determination of its suitable usage for treatment of various diseases related to the immune system.

PBM has well-differentiated immune-modulatory effects on lymphocytes. The light is

*Corresponding author:amela.softic@untz.ba

absorbed mainly by mitochondrial cytochrome c oxidase, leading to increased electron transport, ATP production, and controlled release of nitric oxide and reactive oxygen species. These photochemical events activate intracellular signaling, resulting in changes in protein expression and enhanced cell metabolism. This enhances proliferation of activated lymphocytes and regulates cytokine secretion.[1]

PBA effects are wavelength and dose dependent. Since there are instances where RLT is unsuccessful due to inadequate dosimetry [2,3] and less than optimal choice of treatment parameters [4], further investigation is necessary in order to understand the cause of this occurrence. The light therapy dose is calculated as the product of power density and exposure time [5] and to analyze the effect of different RLT dosage on human peripheral blood lymphocyte's, the samples are monitored at fixed distance for the same light source under different irradiation exposure time parameters.

In the first round of study [6] we investigated the effects of RLT on the proliferation of lymphocytes and showed higher lymphocyte proliferation for all of the irradiated samples. To improve understanding of the overall effect of red/near-infrared light therapy on the cells and its metabolic mechanisms, the study is also extended to the analysis of the RLT impact on cell enzymes synthesis on the same samples.

2 Experimental method

Normally the incubation of lymphocytes lasts 72 hours. Nevertheless, when the first generation mitosis is to be studied, the process can be shortened by stopping cell division with colchicine during the phase of mitosis of cells, which enables us to apply multiple treatments of samples at different times before completing their mitosis cycle [7].

After 24 hours of cultivation, half of the cultures were treated with light emitting diode (LED) source (Figure 1) containing light of 660 nm and a 850 nm light (red and near-infrared wavelengths) in two test intervals of 10 and 20 minutes, and returned to incubation for another 24 hours (samples labeled 48 10m and 48 20 m). Half of these cultures were taken out of the incubator after 48 hours, again irradiated and returned for the remaining 24 hours of incubation, thus being irradiated twice (samples labeled 48+72 10 min and 48+72 20 min).

The remaining part of the cultures was cultivated for 24 hours and first left to incubate for 48 hours and then treated with light at the end of that time interval and returned to additional incubation for 24 hours (samples marked with 72 10m and 72 20m). Furthermore, cultures containing samples that are not treated with red light (control group – samples marked with K) were also in parallel incubated.

Fig. 1 The LED lamp used in the study

To find the total protein concentration in the samples, a colorimetric method is used. It is based on the interreaction of the protein with the appropriate reagent, resulting in a colored product whose concentration is proportional to the protein concentration. The most widely used colorimetric methods (as well as methods for determining proteins in general) is Bradford method. The Bradford method is based on the binding of the dye, Coomassie Brilliant Blue G-250, to the protein molecule which results in a shift in the absorption maximum of the bound dye, relative to the absorption maximum of the unbound dye [8, 9].

The determination is performed relative to a standard line (curve) made with a protein of known concentration where Bovine serum albumin (BSA) typically is used as the standard to increase the signal in samples in spectrophotometric measurements (Figure 2). The amount of the complex formed in solution is defined by the measured absorbance which is proportional to the protein concentration [10]. The maximum absorption spectrum of the dye is at 595 nm. This increase in absorbance at 595 nm is proportional to the amount of bound dye, and consequently to the concentration (amount) of protein present in the sample.

Fig. 2 The set up of the used spectrophotometer

For calibration, a series of dilutions of bovine serum albumin with known protein content was used. For the procedure, the optimal range is 0.01-0.5 micrograms of protein per mL. The response to the required amount of protein depends on the exact pH of the mixture.[11]

In the preparation protocol of the protein extract, lymphocytes culture were centrifuged for 5 minutes at 1000 rpm, and diluted with sodium phosphate buffer in ratio 1:10 and 1:100. When carrying out the procedure, 50 μL of sample and 2 mL of reagent were poured into a disposable plastic cuvette. Everything was mixed well together, and after 5 to 60 min at room temperature, measured at 595 nm in relation to a blank sample in another cuvette (50 μL of sodium phosphate buffer with reagent instead of a sample).

3 Results and discussion

The absorbance of the samples and calibration were measured on a spectrophotometer where the calibration curve (Figure 3.) was directly read for the specified amount of protein in the sample.

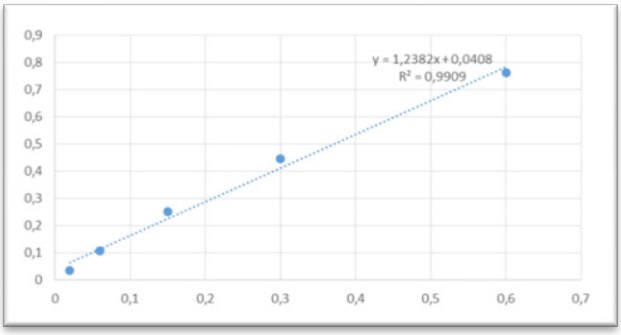

Fig. 3 Bradford calibration curve

The results of the performed measurements of the protein concentrations in lymphocytes are shown in Table 1. The interpretation of the results of the total protein concentration of treated lymphocytes is done in relation to their non-irradiated counterparts (samples marked with K). To make the changes in concentration easier to observe and interpret, the results are also presented in the graphic representation (Figure 3).

Table 1 Measured protein concentration for the test samples

Samples 1:100	Abs 595nm	**P[mg/mL]**
K 48	0,3616	**25,9086**
48 10m	0,3061	**21,4264**
48 20m	0,1911	**12,1386**
K 72	0,2257	**14,9329**
72 10m	0,2062	**13,3581**
72 20m	0,2900	**20,1259**
K 48+72	0,3616	**25,9086**
48+72 10 min	0,4701	**34,6713**
48+72 20 min	0,4757	**35,1236**

As can be seen from the presented data, the protein concentration is altered by irradiation. The results show that repeated RLT has augmented the overall protein concentration in lymphocyte cells. The maximum increase was observed for repeated

exposure, while the results for single exposures led to a smaller increase, or even a decrease in concentration. Namely, for the dilution ratio of 1:100 and a 10 minute exposure time, the concentration for a single exposure was as low as approximately 13 mg/mL, while for the samples with multiple exposures it was over 35 mg/ml (Figure 4).

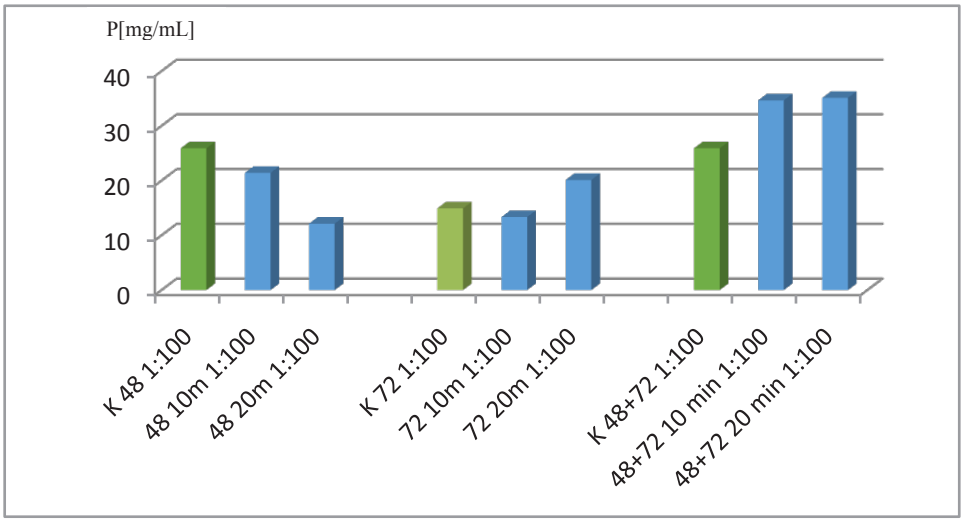

Fig. 4. Graphic representation of the obtained protein concentrations

The obtained results are is in accordance with the observed increased cell proliferation for the same samples from the previous studies [6, 12, 13]. The results evidently demonstrated that the proliferation is altered by irradiation, which ultimately affects the total lymphocyte count, and thus the overall cell protein concentration.
However, it is also necessary to point out that for a couple of instances, especially for the sample treated after 48 hours and single 20 minute exposure, RTL resulted in evident lowering of protein concentration when compared to the controls and other exposure conditions. Since the used method cannot tell if the increased protein concentration has positive or negative connotations, this anomaly may be a sign that the cell death had already started [14, 15] and indicate the need for further investigation of this occurrence.

4 Conclusion

The objective of this study was to investigate the effects of red/infrared light treatment on the total protein concentration in lymphocytes. The interpretation of the obtained results for the protein concentration is done in relation to the unirradiated control cells. Our data showed that the protein concentration is altered by irradiation with maximum increase observed for the repeated exposures.

Since the used method cannot answer whether the increased protein concentration has positive or negative connotations, it is important to carry on the research and extend it to include additional methods that test oxidative stress [16] and changes in the structure of chromosomes. Furthermore, in order to be able to do a statistical processing of data and confirm the beneficial impact of RLT on lymphocyte, the study should be repeated on a larger number of samples.

Also, while the principle for calculating and altering of dose presented in this study is adequate for most uses, light therapy dosing is a much more complex subject. Namely, since the body is 3 dimensional, it should be noted that spatially light therapy dosing requires dose calculation with energy applied to a volume of cells (J/cm^3), rather than on the surface area (J/cm^2), as it is typically measured to simplify the calculation. Thus, further research could as well address this issue.

Declarations

Collection of human tissue samples for this study was approved as part of the study protocol in compliance with ethical standards. This study was performed in accordance with the Declaration of Helsinki. All adult participants provided written informed consent to participate in this study.

Data availability

All data supporting the findings of this study are available within the paper. Data sets generated during the current study are available from the corresponding author on reasonable request.

References

1. K. M. AlGhamdi, A. Kumar, A., N.A. Moussa, Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Lasers in medical science, 27(1) 237–249 (2012) https://doi.org/10.1007/s10103-011-0885-2
2. S. George, M. R. Hamblin, H. Abrahamse, Effect of red light and near infrared laser on the generation of reactive oxygen species in primary dermal fibroblasts. Journal of photochemistry and photobiology. B, Biology, 188 60–68 (2018) https://doi.org/10.1016/j.jphotobiol.2018.09.004
3. M. Devor, What is in a laser beam for pain therapy? Pain;43:139 (1990)
4. H. Chung, T. Dai, S. K. Sharma, Y. Y. Huang, J. D. Carroll, M. R. Hamblin, The nuts and bolts of low-level laser (light) therapy. Annals of biomedical engineering, 40(2) 516–533 (2012) https://doi.org/10.1007/s10439-011-0454-7
5. G. Sajan,Complete guide to light therapy dosing, https://redlightman.com/blog/complete-guide-light-therapy-dosing, Accessed 11 July 2025
6. A. Softić, S. Cipurković, S., A. Avdić, A. Effects of 660 nm red light and 850 nm near infrared light on human blood lymphocytes. International Physics Conference in Bosnia and Herzegovina, 4(4), 43. (2024)
7. Z. Mujkić Ispitivanje citotoksičnosti bisoprolola na kulturi humanih limfocita, Master Thesis, University of Sarajevo (2022)
8. Niketić, Uputstva za vežbe iz biohemije za student hemije smera istraživanje i razvoj, Beograd, Univerzitet u Beogradu (2007)
9. S. J. Compton, C..G. Jones, Mechanism of dye response and interference in the Bradford protein assay. Anal Biochem. 151(2), 369-74 (1985)

10. S. Ge, K. Kojio, A. Takahara, T. Kajiyama, Bovine serum albumin adsorption onto immobilized organotrichlorosilane surface: influence of the phase separation on protein adsorption patterns. J. Biomater. Sci. Polym. Ed. 9(2), 131-150 (1998)
11. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Analytical Biochemistry, 72 (1-2), 248-254 (1976)
12. C. Webb, M. Dyson, W. H. Lewis, Stimulatory effect of 660 nm low level laser energy on hypertrophic scar-derived fibroblasts: possible mechanisms for increase in cell counts. Lasers in surgery and medicine, 22(5) 294–301(1998). https://doi.org/10.1002/(sici)1096-9101(1998)22:5<294::aid-lsm6>3.0.co;2-k
13. A. Pereira, C. Eduardo, E. Matson E, M. Marques, Effect of low-power laser irradiation on cell growth and procollagen synthesisof cultured fibroblasts. J Clin Laser Med Surg 31: 263–267 (2002)
14. L. Frigo, G. M. Fávero, H. J. Lima, et al, Low-level laser irradiation (InGaAlP-660 nm) increases fibroblast cell proliferation and reduces cell death in a dose-dependent manner. Photomedicine and laser surgery, 28 Suppl 1, S151–S156 (2010). https://doi.org/10.1089/pho.2008.2475
15. A. Casas, C. Perotti, H Fukuda, A.M. Batlle, A. M. Photodynamic therapy of activated and resting lymphocytes and its antioxidant adaptive response. Lasers in medical science, 17(1), 42–5, (2002). https://doi.org/10.1007/s10103-002-8265-6
16. M. K Giacci, Hart, Hartz N. S., R. V., Harvey, A. R., S. I.Hodgetts , M. Fitzgerald, Method for the assessment of effects of a range of wavelengths and intensities of red/near-infrared light therapy on oxidative stress in vitro. Journal of Visualized Experiments: JoVE, (97), 1-8. Article e52221. (2015). https://doi.org/10.3791/52221