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THE EFFECTS OF RED/INFRARED LIGHT TREATMENT ON THE TOTAL PROTEIN CONCENTRATION IN LYMPHOCYTES

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Introduction

The effectiveness of red light therapy (RLT) on lymphocytes is of importance in determination of suitable usage for treatment of various diseases related to the immune system.
Since there are instances where low level laser therapy is unsuccessful due to inadequate dosimetry [1] and lack of studies comparing treatment parameters [2], further investigation is needed.
The light therapy dose is calculated as the product of power density and exposure time [3] and to analyze the effect of different RLT dosage on human peripheral blood lymphocyte's, the samples are monitored at fixed distance under different irradiation exposure time parameters.
In the preliminary study [4] we investigated the effects of RLT on the proliferation of human lymphocytes and showed higher lymphocyte proliferation for all of the irradiated samples. To better understand overall effect on the cells and the metabolic mechanisms involved in red/near-infrared light therapy, the study is also extended to the analysis of the RLT impact on cell enzymes synthesis on the same samples.

Methods

To determine the total protein concentration in the samples, a colorimetric method is used. Its determination is base on the (inter)reaction of the protein with the appropriate reagent, resulting in a colored product whose concentration ("color intensity") is proportional to the protein concentration. The used Bradford method is a colorimetric method for determining protein concentration 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 free, unbound dye [5].

The determination is performed based on a standard line (curve) made with a protein of known concentration. For calibration, a series of dilutions of bovine serum albumin with known protein content was used. The optimal range of this procedure is in the interval 0.01-0.5 micrograms protein/mL sample. The absorbance of the samples and calibration were measured on a spectrophotometer (Fig 1.) at 595 nm. The calibration curve was immediately read for the specified amount of protein in the sample (Figure 2.) The increase in absorbance at 595 nm is proportional to the amount of bound dye, and thus the amount (concentration) of protein present in the sample.

Fig. 1. The set up of the used spectrophotometer

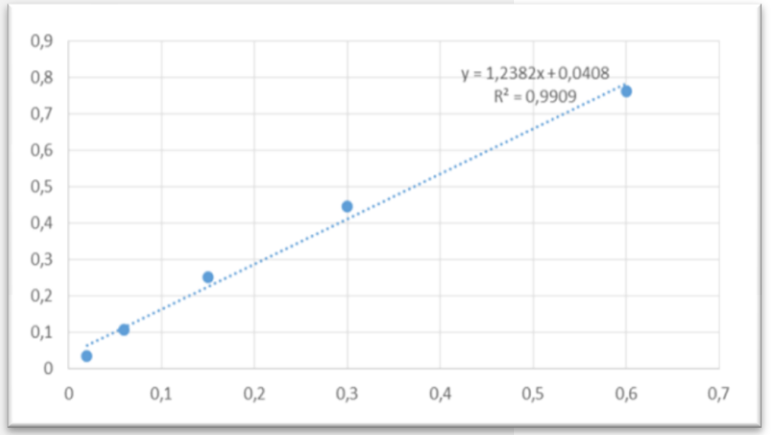

Fig. 2. Bradford calibration curve

Results and disscusion

The interpretation of the results of the total protein concentration of irradiated lymphocytes is done in relation to their non-irradiated counterparts – controls (samples marked with K).

As can be seen from the presented data (Table 1., Fig. 3.), the concentration is altered by irradiation. A maximal increase was observed with repeated exposure, while the results for a single exposure led to lower values. For instance, for the non-irradiated samples the concentration, for a single exposure was as low as 12 mg/mL, while for the samples with multiple exposure it was more than 30 mg/ml. This is accordance with the previously observed cell count for the same samples.

It is also necessary to note that the single treatment after 48 hours, when compared to the controls, resulted in lowering of concentrations which indicates the need for further investigation for the reasons of this occurence and fine-tuning of the experimental set-up.

Table 1. Protein concentration for the samples

Samples	Abs 595nm	P[mg/mL]
K 48 1:100	0,3616	**25,90858**
48 10m 1:100	0,3061	**21,42626**
48 20m 1:100	0,1911	**12,13859**
K 72 1:100	0,2257	**14,93297**
72 10m 1:100	0,2062	**13,3581**
72 20m 1:100	0,29	**20,12599**
48+72 10 min 1:100	0,4701	**34,6713**
48+72 20 min 1:100	0,4757	**35,12357**

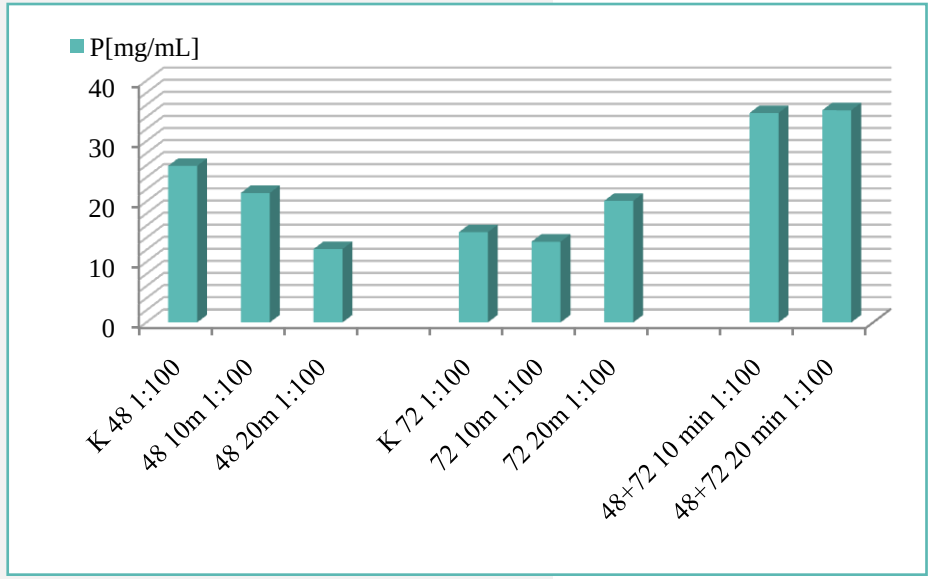

Fig. 3. Graphic representation of the measured protein concentration

Conclusion

The objective of this study was to investigate the effects of red/infrared light treatment on the total protein concentration in lymphocytes. The obtained results clearly imply that the protein concentration is augmented when multiply treated with red/IR light.

However, 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.

References

1. Sajan, G.,Complete guide to light therapy dosing, https://redlightman.com/blog/complete-guide-light-therapy-dosing/
2. Giacci, M. K., Hart, N. S., Hartz, R. V., Harvey, A. R., Hodgetts, S. I., & Fitzgerald, M. (2015). 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. https://doi.org/10.3791/52221
3. Softić, A., Cipurković, S., & Avdić, A. (2024). 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.
4. Niketić, V. (2007).Uputstva za vežbe iz biohemije za student hemije smera istraživanje i razvoj, Beograd, Univerzitet u Beogradu
5. Compton S.J., Jones C.G.(1985). Mechanism of dye response and interference in the Bradford protein assay. Anal Biochem. 151(2), 369-74