Effect of 650 nm Diode Laser on the Cross-Bonding Formation of Human RBC Membrane

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Abstract

Background & Objective(s): - The current study was designed to study the effect of diode laser on red blood cell membrane protein indirectly assessed (by membrane cross bonding formation).

Materials & Methods: - The packed cells were washed three times in $M_{300,}$ and then were resuspended in M_{600} at an approximate haematocrit of 2%. The RBCs suspension was divided into four equal aliquots. One aliquot was served as control. The remaining three aliquots were irradiated by laser for 30, 40 and 60 min. after each specific time, the control and the irradiated samples were centrifuged and the harvested RBCs were washed once in M_{200} and were resuspended at an approximate haematocrit of 0.2% in M_{200}. The percentage of cross bonded cells was determined microscopically in each sample as described later.

Results: - It was found that the exposure of red blood cells to the laser for 30 min didn't induce membrane cross- bonded formation. However, prolongation of the irradiation time for 40 min and 60 min was associated with a significant increase in the percentage of cross bonded cells.

Conclusion:- exposure of red blood cells to low level diode laser (λ= 650 nm, w= 50 mW) for long period of time equal or more than 40 min led to denaturation of the membrane protein of red blood cells.

Keywords:- Laser + Red Blood Cells +Membrane Protein + Cross-Bonding

Introduction

The red blood cell membrane is composed of a lipid bilayer and membrane skeleton that underlies the bilayer and associated with it through protein-protein and protein-lipid interactions. The key constituents of the red blood cell membrane skeleton are spectrin, actin, band 4.1 and 4.9. Spectrin is the most abundant and most important skeletal protein [1].

Membrane cross bonding occur when promote an adhesion between opposing areas of the cytoplasmic face of the red cell membrane. To produce membrane cross bonding, internal membrane contact is required either during the treatment or after ward. Membrane contact in the dimple region was achieved by cell shrinking in hypertonic medium [2].

Membrane cross bonding or membrane-bridge become visible microscopically when the cells are swollen in a hypotonic medium and these bridges are strong enough to resist the membrane tension occurring at osmotic swelling [3]. Fischer [1988] concluded that the spectrin provides the molecular crosslink in membrane cross-bonding.

Lasers as highly stable source of coherent and monochromatic light have been used extensively in technical and medical applications.

The effect of laser irradiation on biological objects depends on experimental conditions such as the type of cells irradiated, wave length and intensity of light, etc. [4].

High energy laser irradiation causes destruction and vaporization of tissues, which has been exploited in surgery [4]. On the other hand a positive effect of low-energy laser irradiation with red light on regeneration has been found in various tissues, such as skin [5], bone [6], and nerves [7]. However, the molecular mechanisms of laser-induced changes in cell structure and function remain unclear [4, 8, 9, 10, 11, 12].

Efforts have been focused on studies of light dependent changes in various biological objects, such as blood components [5]. It is well known that the cell membrane is the first line of interaction between cellular machinery and the outside world. It is the site of key event in laser interaction with cells [9]. Among the latter, red blood cell membranes are most prominent because of their simplicity, availability and physiological importance [4]. A variety of studies both in vivo and in vitro showed significant influence of laser irradiation on red cells functional state [12]. At the same time another group of workers found not detectable effects of laser exposure [12].

The present study was design to investigate if the low power diode laser (650nm) able to induce changes in membrane protein by cross bonding formation.

Materials & Methods

*** Sample collection:-**

Fresh blood samples (5ml) were collected from apparently normal healthy donors by venipuncture in an EDTA tube (1mg/ml of blood). Blood sample was processed immediately.

*** Suspending media:-**

Suspending media are designated by M carrying as a subscript their Osmolarity in mOsm. The isotonic solution (M_{300}) contains (mM) Kcl 150 and Tris-Hcl 10. The hypotonic solution (M_{200}) contains (mM) Kcl 100 and Tris- Hcl 10. The hypertonic solution (M_{600}) contains (mM) Kcl 150, Tris-Hcl 10

and sucrose 300. The PH of all solutions was adjusted to 7.4.

*** Laser set up:-**

The device that has been used in this work was low-power diode laser source, emitting red light at a wave of length of 650 nm with output power 50 mw. The laser beam was directly delivered to blood samples in tubes with a (5mm) diameter irradiation spot, and the irradiation time was 30, 40 and 60 min. Thus, the power density was (50mW/ 0.78 cm) = 63.69×10^{-3} W/ cm^2.

*** Processing and irradiation of blood samples:-**

The anticoagulated blood sample was centrifuged at x 1500g for 5min, the plasma and the buffy coat were discarded by gentle aspiration and the packed cells were washed three times in M_{300}. The supernatant was carefully removed after each wash.

The freshly collected washed packed RBCs were then resuspended in M_{600} at an approximate haematocrit of 2%. The RBCs suspension was divided into four equal aliquots. One aliquot was served as control. The remaining three aliquots were irradiated by laser for 30, 40 and 60 min. after each specific time, the control and the irradiated samples were centrifuged and the harvested RBCs were washed once in M_{200} and resuspended at an approximate haematocrit of 0.2% in M_{200}. The percentage of cross bonded cells was determined microscopically in each sample as described below.

*** Counting the cross bonded cells:-**

The technique used in the present study for counting the cross-bonded cells was previously published by Fischer [1986, 1988], in which RBCs were suspended in a hypertonic solution to induce a contact between the two sides of the RBC membrane protein. If cross-bonding formation was taking place, single or multiple hollows can be seen under microscope upon suspending the cells in a hypotonic solution. These hollows are the sites of the cross-bonded areas between the two sides of membrane proteins.

Random samples were selected (to count the cross-bonded cells) by positioning filed to be counted according to a fixed scheme. In this study, the Neubauer counting chamber was used for counting cross-bonded cells.

For counting the cross-bonded red cells after laser irradiation, a drop of the red cells suspension in M_{200} was placed on a Neubauer counting chamber and examined by phase contrast microscopy (40 × objective), (figure 1).

Two medium size squares on the counting chamber were chosen to count the cross-bonded cells in all the samples studied. Then the percentage of the cross-bonded cells was determined.

*** Statistical analysis:-**

All values are means ± SD. The differences were assessed by student paired t-test. $P<0.05$ was considered to be statistically significant.

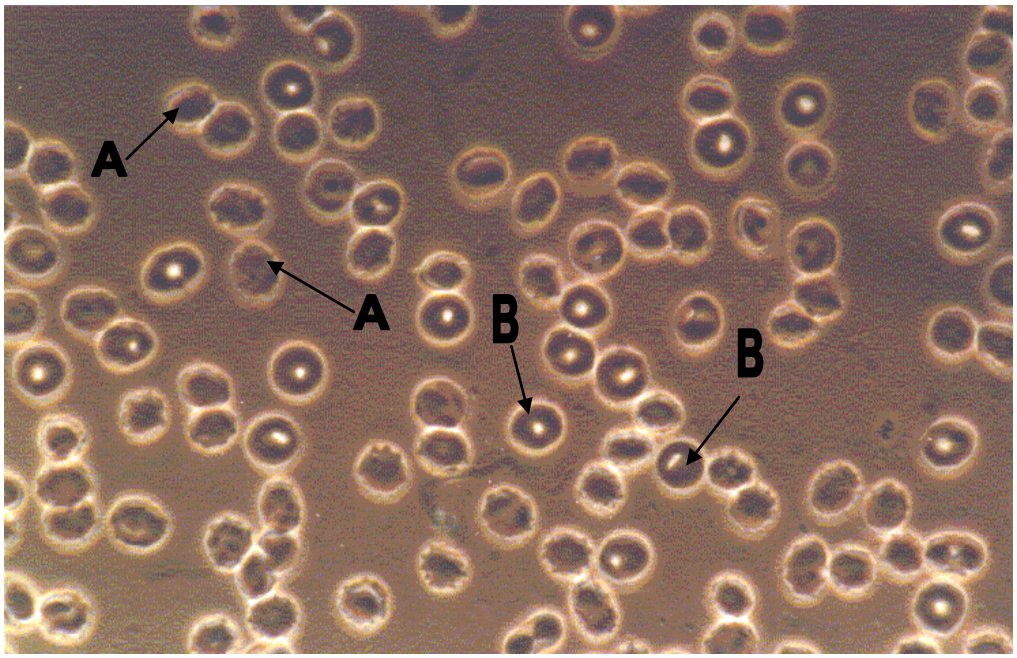

Figure (1): RBCs as shown under phase contrast microscope (x 40 objective) in M_{200} solution .A= Normal cell. B= Cross– bonded cell.

Results

The percentage of cross-bonded cells which were irradiated by laser for 30 min was similar to the percentage of cross-bonded cells which were represented as control. Prolongation of the irradiation time for 40 min led to formation of membrane cross-bonding in a significant number of RBCs (6.13±2.3%, n=12) in comparison with unirradiated cells.

Moreover, irradiation of RBCs, for 60 min was associated with a significant increase in the percentage of cross-bonded RBCs (23.71±4.58%, n=12) in comparison with control cells **(figure 2).**

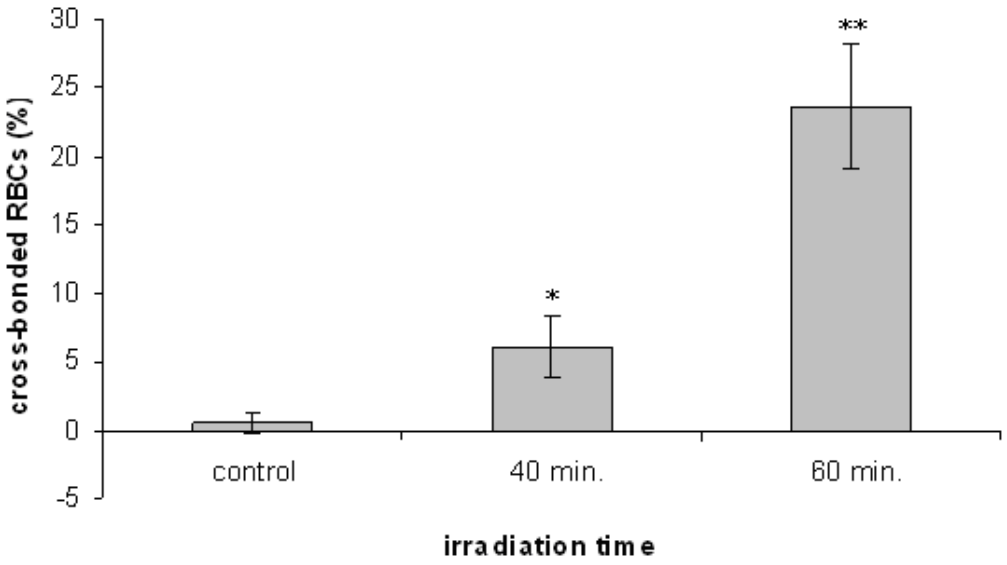

Figure (2): Effect of irradiation time on the percentage of cross-bonded RBCs irradiated by Diode laser. Each value represents mean + SD of twelve experiments for 40 and 60 min. irradiation time. *=P<0.0001, **=P<0.0001(by paired t-test) compared to non-irradiated RBCs (control).

Discussion

The present research aimed to investigate the ability of low power diode laser to form membrane cross bonding in membrane protein of red blood cells.

The effect of laser irradiation on RBCs has been studied by several investigators and various contradictory results have been reported. Yova, D et al [1994] found that the RBCs deformability was decreased after He-Ne laser continuous irradiation (632.8nm, 0.5mW, during 5min). Viscor et al [1989] have detected a lower RBCs deformability and higher osmotic fragility of laser exposed erythrocytes (632.8nm, 7mW, irradiation time 10 min). In addition, Kujawa et al [2004] concluded that, near- infrared laser light radiation (810nm) induced long term conformational transitions of red blood cell membrane which were related to changes membrane proteins and lipid bilayer. In contrast, Wilander et al [1986] did not detect differences in hemoglobin content, absorbance of single erythrocyte at 555nm and in cell shape due to the individual exposure of RBCs to He- Ne laser.

The present study showed for the first time that membrane cross-bonding occurred when red cells were irradiated by low level diode laser (650nm wave length, 50 mW power); no previous publications indicated that laser is able to induce membrane cross-bonding formation.

From the data of the present study, exposure of red blood cells to low level laser for 30min did not induce any change in cells (i.e. there is no cross bonded cells was formed) while irradiation of red blood cells by laser for 40 min led to formation of membrane cross bonding in a significant number of RBCs (fig.2) and the percentage of cross bonded

cells further increased with increasing irradiation time from 40 min to 60 min. This result is in parallel with the result of Singh and Vatsala [1979] who were able to reveal noticeable functional and morphological alterations such as an increase in aggregability, crenation and hemolysis in RBCs treated with He-Ne laser (400-800 mJ/cm^2) for 15 and 30min.

Pervious studies concluded that the membrane cross bonding technique is a valid and quantitatively sensitive to detect changes in membrane proteins and could be utilize as simple useful tool to test for the changes of red cell membrane protein [1].

It is possible to suggest that the exposure of red blood cells to diode laser (λ= 650nm, W=50mW) for long period of time more than 40 min led to denaturation of the structural membrane proteins.

Conclusion

1-Exposure of red blood cells to low level diode laser (λ = 650nm, W=50mW) for long period of time more than 40min lead to formation of membrane cross bonded cells, means lead to denaturation in membrane protein.

2-Exposure of red blood cells to low level diode laser (λ= 650nm, W=50mW) for time of irradiation less than 40 min did not induce membrane cross bonding formation means did not affect on membrane protein of red blood cells.

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References

1-AL- Gailani, B.; Musa, T. and AL- Yasiri, A.: Validation and evaluation the cross bonding technique to detect the changes in cell membrane protein. Iraqi journal of community medicine. (2001), Vol. 14(2), pp.176-182.

2-Fischer, T.: Role of spectrin in cross-bonding of the red cells membrane. Blood cells. (1988), Vol. 13, PP. 377- 394.

3-Fishcer, T.: Transcellular cross-bonding of the red blood cell membrane. Biochimica et Biophysica Acte. (1986), Vol. 861, PP. 277-286.

4-Kassak, P.; Sikurova, L.; Kvasnicka, P., and Bryszewska, M.: The response of Na^+/ K^+-ATPase of human erythrocyte to green laser light treatment. Physiological research. (2006), Vol. 55, PP. 189-194.

5-Conlan, J.; Rapley, W. and COBB, M.: Biostimulation of wound healing by low energy laser irradiation. A review. J Clin. Periodontal. (1996), Vol. 23, PP. 492-496.

6-Yaakobi, T.; Maltz, L. and Oron, V.: Promotion of bone repair in the cortical bone of the tibia in rats by low energy laser (He–Ne) irradiation. Calcif tissue int. (1996), Vol. 59, PP. 297-300.

7-Assia, E.; Rosner, M.; Belkin, M.; Solomon, A. and Schwartz, M.: Temporal parameters of low energy laser irradiation for optimal delay of post traumatic degeneration of rat optic nerve. Brain research. (1986), Vol. 476, PP. 205-212.

8-Yanhong, C.; Zhouyi, G.; Yanping, Z.; Ying, Z.; Yanfang, Q.; Jiye, C. and Songhao, L.: Reactive effect of low intensity He- Ne laser upon damaged ultrastructure of human erythrocyte membrane in fenton system by atomic force microscopy. Acta biochimica et biophysica Sinica. , (2007), Vol. 39, PP. 484-489.

9-Yova, D.; Haritou, M. and Koutsoruis, D.: Antagonistic effects of epinephrine and helium. Neon (He-Ne) laser irradiation on red blood cells deformability. Clin. Hemorheol. (1994), Vol. 14, No.3, PP.369-378.

10-Xian- Qiang, M.; Chen, J.; Junliang, Z. and Welzhou, L.: In vitro effects of helium. Neon laser irradiation no human blood: Blood viscosity and deformability of erythrocytes. Photomedicine and laser surgery. (2004), Vol. 22, No. 6, PP. 477-482.

11-Kujawa, J.; Zavodnik, Le. Zavodnik, LL. and Bryszewska, M.: Low-intensity near-infrared laser radiation induced changes of acetylcholinestrase activity of human erythrocytes. Journal of clinical laser medicine and surgery. (2003), Vol. 21(6), PP. 351-355.

12-Kujawa, J.; Zavodnik, L.; Zavodnik, I.; Buko, V.; Lapshyna, A. and Bryszewska, M., Effect of low intensity (3.75-25J/cm^2) near infrared (810nm) laser radiation no red blood cells ATPase activities and membrane structure. Journal of clinical laser medicine and surgery. (2004), Vol. 22(2), PP. 111-117.

13-Viscor, G.; Palacios, L. and Palomeque. J.: Affect of He- Ne laser irradiation of blood in vitro no erythrocyte microrheological properties. Laser life sciences. (1989), Vol. 3(1), PP 31-36.

14-Wilander, L.; Rundquist, P.A.; Oberg, P.A. and Odman, S., laser He-Ne light exposure of red blood cells. Medical Biological Engineering computer. (1986), Vol. 24, PP. 558-560.

15-Singh, M. and Vatsala, T.M.: Helium- Neon laser induced changes in erythrocytes. Curr. Sci. (1979), Vol. 48, PP. 720-721.

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