

SHORT COMMUNICATION

CERTAIN IMMUNO-HAEMATOLOGICAL EFFECTS
OF LASER IRRADIATION

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Summary : The effects of low power He-Ne laser of 7 milliwatt power on certain immuno-haematological parameters were studied using fresh human blood samples. The parameters studied include electrophoretic mobility of haemoglobin, quantification of immunoglobulins, soluble immune complex levels, blood grouping, Rh typing and neutrophil function tests.

Our results show that there is no significant change in the parameters studied after laser irradiation for 60 minutes except changes in the electrophoretic mobility of haemoglobin. In addition laser exposure causes haemolysis in all the samples examined.

Key words : Low power Laser
phagocytosis

immune complex
haemoglobin

candida
electrophoresis

INTRODUCTION

The word laser is an acronym for "Light Amplification Stimulated by Emission of Radiation" and involves an emission process of tremendous energy photons producing an intense beam of electromagnetic radiation in the form of monochromatic light (1). The use of Laser has permeated into various fields of medicine and surgery as well as in other fields of science and technology. This widespread use of laser has given rise to problems of safe dosimetry due to its varied effects on life systems. Low power lasers used in clinical medicine and biomedical research is said to have very little unfavourable effects on biological systems, but an indepth comprehensive studies on the effects of laser on immuno haematological system are lacking. The effects of laser impact on a tissue are highly varied and most of them are molecular in nature. These changes are difficult to assess by routine physical methods.

The present in vitro study was undertaken to elucidate these changes using standard techniques which can be carried out in a standard Physiology/Biochemistry laboratories.

MATERIALS AND METHODS

Fresh venous blood from healthy volunteers and donors were collected with and without anticoagulant in sterile glass tubes and divided into two parts. One part served as control and the other part was exposed to a collimated beam of He-Ne laser with 7 milliwatt power at a temperature of 25°C for 60 minutes in a specially designed petri dish. The total exposure energy was 5.5 joules/cm². In the control and laser exposed samples the following parameters were studied.

Hemoglobin Electrophoresis : Haemolysates were prepared using fresh blood samples (2) and divided into two parts - the control and test samples. The

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test samples were exposed to laser for 60 minutes as described earlier. Electrophoresis was carried out using these samples on agarose gel with phosphate buffer at pH 6.25 for 90 minutes, at the end of which the slides were stained with Amido black, fixed and labelled. The distance between the origin and distal end of the band was measured in millimeters and the values for the controls and exposed samples were compared in 15 different samples of blood.

Quantification of Immunoglobulins: The immunoglobulins in the serum were quantified using Single radial immuno diffusion technique (3). Controls and laser exposed serum samples were injected into SRID plates obtained commercially (Immunodiagnosics, India). Standard reference sera serially diluted was also injected at the same time. The plates were incubated at room temperature over night for the development of precipitin rings. A standard serum and the values of the samples were interpolated on the standard graph. Immunoglobulins were quantified in 15 different blood samples.

Soluble immune complex levels were estimated in 20 different blood samples using polyethylene glycol precipitation method (4).

Blood grouping and Rh typing were also done in 10 different samples of blood.

Candida phagocytosis by neutrophils was studied using buffy coats obtained from control and laser exposed blood samples (5). Phagocytic index (Percentage of neutrophils which have ingested candida) and avidity index (average number of candida within a neutrophil by counting candida in 100 neutrophils) were determined in 15 different blood samples.

RESULTS

The mean distance of movement of Hemoglobin for control samples was 18.7 mm and that of the

laser exposed samples was 21.7 mm. Statistically this difference is significant at 10% level ($P < 0.01$).

Levels of immunoglobulins and soluble immune-complexes did not show any change after the laser irradiation.

Both the control and laser irradiated samples reacted in the same manner with their respective blood group antisera.

No significant change was seen in the phagocytic index and avidity index in control and laser exposed samples.

All the laser exposed samples showed evidence of haemolysis. No alterations in the leucocyte morphology was noticed although the number of red cell ghosts were seen in all the laser exposed samples.

DISCUSSION

The fundamental action of laser is due to the absorption of light energy by the tissues resulting in heating of the tissue. In addition to the thermal effect, pressure recoiling, elastic waving and electromagnetic field changes also influence the tissues and bring about molecular changes in the affected tissues (1). Pigmented tissues are known to absorb the laser energy better (6, 7, 8, 9). As hemoglobin is a coloured substance with complex molecular structure, laser impact and absorption might bring about changes in its molecular structure and also changes in the electrical charges on the hemoglobin molecule. The electrophoretic study was undertaken specifically to elucidate this effect, the increase in the electrophoretic mobility of hemoglobin noticed in this study may be attributed to certain molecular changes induced by laser impact.

Since the laser impact may alter the chemical structure of the molecules which determines the immuno reactivity of the proteins, the study of

quantification of immunoglobulins by SRID were undertaken. Though similar studies could also have been done with serum albumin, the immunoglobulins were selected because the ready made SRID plates for quantification of immunoglobulins were readily available in the market. The fact that no change was noticed in the reactivity of immunoglobulins with specific anti sera after laser impact does not exclude the possibility of laser action since the site of damage may be else where other than the immuno reactive sites, and if so no quantifiable change could be observed by this method.

Immune complexes in the blood are antigen antibody complexes which could be influenced by laser action resulting in accelerated dissociation of the complexes. Our results show no change in the levels of these complexes after laser irradiation indicating thereby that either the energy levels involved is insufficient to dissociate the immune complexes or laser has no specific capability to dissociate the antigen antibody complexes. Laser is known to destroy the red cell membrane resulting in haemolysis (10, 11). Such change may also involve antigenic sites on the cell membrane resulting in alteration in the specific reaction of antigen with the

corresponding antibody (9). Irradiation of blood samples with laser has not resulted in any such effect.

Candida phagocytosis by neutrophils was also unaffected by laser irradiation showing thereby that white cell membrane is more resistant to laser irradiation than the red cell membrane. Further, the fact that there is no change in the white cell count after laser irradiation (not reported in this paper) supports this concept.

However the changes in the antigenicity of blood group substances, and changes in white cell morphology and mobility as shown by other workers (12, 8, 1) be attributed to the higher powers of lasers used and different wave lengths employed.

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