Effect of Laser on Phagocytic Activity of Polymorphoneutrophils

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Fac Med Baghdad 2010; Vol. 52, No. 3
Received Dec.2009
Accepted Feb. 2010

Summary:

Background: It is well known that the low level laser irradiation has act on immune system cells in a number of ways, one of them includes increasing phagocytic activity of irradiated cells. This study was established to shed light on the possible effect of laser irradiation on phagocytic activity of polymorphoneutrophils.

Subjects and methods: Fresh blood samples were obtained from twenty healthy volunteers for phagocytosis assay. The polymorphoneutrophils were isolated from blood and examined their phagocytic capacity befor and after exposure to laser irradiation.

Results: The present study revealed a significant increase in the mean percentage of phagocytosis after exposure to diode laser of wave length (632) nm (red), which was further increased by increasing the time of exposure.

Conclusion: The current study suggests that low level of laser can increase the phagocytic activity of polymorphoneutrophils, and this increase propotional to increaseing the time of exposure.

Key words: Laser irradiation, PMNs, Phagocytosis.

Introduction:

Laser irradiation has been found to modulate various biological processes. It is a beneficial clinical modality in enhancing the process of wound healing (1), and inflammatory suppression (2). Several animal studies have shown that low power laser irradiation modulates immune cell functions, such as cell-mediated hypersensitivity reactions and inflammatory process (3). Laser light also influence blood cells, irradiation of red blood cell lysate with a neonium laser (337 nm) induces oxidation of hemoproteins (4) Polymorphoneutrophils (PMNs) are one component of blood known to mediate its phagocytic biological action via generating substantial amount of nitric oxide (NO) and reactive oxygen species (ROS) upon exposure to external stimuli (3) including laser light (5). The granular apparatus of these cells possesses high bactericidal potential and is involved in the activation of phagocytized microorganisms (6). The strong and growing interest in studying of mechanisms of the low power laser irradiation action on inflammation-related functions of phagocytic cells has been marked. One study had shown that laser irradiation can modulate the phagocytosis of particles and production of ROS by leukocytes (7). Other study showed the effect of laser generated oxygen free radical in biological system (8).

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It was suggested that the quantity of generated free radical depends on the power of the laser being used (7). The ability of PMNs to ingest yeast particles is the criterion commonly applied in studies of their phagocytic capacity and this study was established to shed light on the possible effect of laser light on phagocytic activity of PMNs.

Subjects and methods:

Phagocytosis was determined according to the method of Cech and Lehrer (9).

Preparation of PMNs Suspension

Blood samples (5ml) were collected from twenty healthy subjects in heparinized tube (heparin con.5000iu\ml),and then incubated at 37ċ for 1 hr, after erythrocytes sedimentation the leukocyte-rich plasma was removed by sterile pipette in to other tube, then washed twice with RPMI-1640 and resuspended in the same medium to give a final concentration of (1×10^6) cells/ml.

Heat Killed Candida albicans Suspension

The suspension was prepared as describedby Wilkinson and Chan & Baltish (10&11).

The yeast cultured in 30ml of subouroude dextrose broth in glass universal tube with screw cap, incubated in orbital incubator at 37ċ for 24 hr, then centrifuged at 3000 rpm for 15 min. The pellet was suspended with Hank's balanced salt solution (HBSS), then placed in boiling water bath for 30 min. The yeast was counted by haemocytometer, diluted to (5×10^6) cells/ml.

Phagocytosis assay

* 0.25 ml of PMNs suspension was mixed with 0.25 ml of yeast suspension, 0.25 ml of HBSS and 0.25

ml of normal human serum (blood groupAB) in sterilized test tube.

* The mixture was incubated at 37ċ for 30 min, and then centrifuged at 1000 rpm for 5 min. The pellet was gently resuspended, one drop was placed on a slid and smeared, then left to dry, fixed by methyl alcohol (99%) for 10 min and stained for 20 min with Geimsa stain. then, examined under oil immersion and at least 200 cells of PMNs were counted, then the percentage of phagocytosis were found as follow:

Phagocytosis Factor = No. of phagocytic PMNs× 100
Total No.

Laser irradiation

PMNs were irradiated by diode laser (lambda, jenjaing com., Taiwan) of (632) nm in wave length (red), in continuous mode. The spot size was $6mm^3$ and the power density was $1mw/mm^3$; the duration time was 15 min and 30 min.

Statistical analysis

Statistical analysis involved calculations of mean, median, standard deviation (SD), standard error (SE) and student t-test.

Results:

Blood samples were collected from twenty healthy volunteers for phagocytosis assay before and after laser irradiation. The current study revealed significant increase ($p<0.005$) in the mean percentage of phagocytosis after exposure to irradiation for 15 min as shown in table-1.

Table 1: The difference in mean percentage of phagocytosis befor and after exposure (15 min).

	Before exposure	**After exposure (15)**
Percentage of phagocytosis		
Mean	72.4	75.2
Median	72	74
SD	1.3	2.52
SE	0.29	0.56
NO	20	20

p<0.005

Moreover, table-2 showed a high significant differences ($p<0.001$) in the mean percentage of phagocytosis after irradiation for 30 min. There was a significant differences in the mean of phagocytic activity of PMNs when the time of exposure to laser was increased from 15 min to 30 min ($p<0.001$), (table-3).

Table 2: The difference in mean percentage of phagocytosis befor and after exposure (30 min).

	Before exposure	**After exposure (30)**
Percentage of phagocytosis		
Mean	72.4	77.9
Median	72	79
SD	1.3	4.12
SE	0.29	0.92
No.	20	20

p<0.001

Table 3: The effect of increasing the exposure time on the mean percentage of phagocytic activity of PMNs.

	After exposure (15)	**After exposure (30)**
Percentage of phagocytosis		
Mean	75.2	77.9
Median	74	79
SD	2.52	4.12
SE	0.56	0.92
No.	20	20

p<0.001

Discussion:

Neutrophils play a major role in host defence via the phagocytosis and destruction of pathogens during acute inflammation. The binding of opsnized microorganisms or immune complexes to neutrophil immunoglobulin receptors (12) can activate a number of processes such as phagocytosis, degranulation and activation of the NADPH oxidase(13). The results of the present study was comparable with other results (14, 15&16), Dolgushin and Gizinger mentioned that the irradiation by low power laser light increased activity of phagocytosis, so they concluded that low power laser irradiation normalized disturbed neutrophil function (14). Other experimental study have shown that there is immune corrective effect of different methods of low power laser therapy in the exacerbation period and more essential decrease of *Helicobacter pylori* microbial contamination in patients with peptic and duodenal ulcer (17). The effect of laser irradiation on the biological systems are due to the presence of acceptors that can absorb visible light (3). In the blood system these acceptors are presented by various forms of hemoglobulin, catalase, cytochrome b, cytochrome oxidase and peroxidase . In conclusion the current study suggessthat low power of laser can increase the phagocytic activity of PMNs, which was further increasing by increase the time of exposure.

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