Journal of Innovative Optical Health Sciences
Vol. 1, No. 2 (2008) 285–294

World Scientific
www.worldscientific.com

EFFECTS OF RED LIGHT AT 640 nm FROM LIGHT EMITTING DIODES ON THE RESPIRATORY BURST OF HUMAN NEUTROPHILS

MIN WU*,†, LING ZHU*,†,‡, BINA HU*, TIMON CHENG-YI LIU*,†,§, DONG-LIANG RONG* and TONG-SHENG CHEN†

*Laboratory of Laser Sports Medicine
South China Normal University
Guangzhou, GD 510006, China

†MOE Key Laboratory of Laser Life Science
South China Normal University,
Guangzhou, GD 510631, China
§liutcy@scnu.edu.cn

Photobiomodulation (PBM) has been reported to have effects on respiratory burst of polymorphonuclear neutrophils (PMNs), but little focus was on the individual differences of human PMNs. The latter was investigated in this study. The PMNs were isolated from peripheral blood of 13 volunteers (10 ordinary persons, 3 athletes) and treated by red light (640 ± 15 nm) from light emitting diodes (RLED) at 50, 100, 300, 500 and 1000 J/m^2 for 100 seconds, respectively. Blood samples of athletes were extracted at different running stages in 10 km non-interrupted long-distance running, before running, 1 hour after running began, just finishing the running, resting for 1 hour and 2 hours after running. The PMN respiratory burst was assessed by the nitroblue tetrazolium test. It was found that there were three types of RLED PBM on the respiratory burst of 3 types of PMNs, respectively, inducing for the subactivated PMNs, inhibiting for the overactivated PMNs and none for the PMNs in homeostasis. It was then concluded that there may be RLED PBM on dysfunctional human PMNs while none on those in homeostasis, and RLED at 300 J/m^2 for 100 seconds may have bi-direction modulation on PMN respiratory burst.

Keywords: Polymorphonuclear neutrophils; photobiomodulation; homeostasis.

1. Introduction

Polymorphonuclear neutrophils (PMNs) represent 50% to 60% of the total circulating leukocytes and stand at the "first line of defense" against pathogenic microbial intruders that penetrate the body's physical barriers.[1] PMNs are essential for host defense. These cells are one of the main effector cells in the innate natural (nonspecific) immune system. Their major role is to phagocytose and destroy

‡Ling Zhu has the same contribution to this paper.
§Corresponding author.

infectious agents but they also limit the growth of some microbes, thus buying time for adaptive (specific) immunological responses to develop.[2] There are two kinds of antimicrobial activities of PMNs, reactions initiated by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in phagosomes and reactive oxygen species (ROS) — induced formation of PMN extracellular traps (NETs).[3,4] The photobiomodulation (PBM) of NADPH oxidase mediated respiratory burst will be studied in this paper.

PBM, once called biostimulation, is characterized by inducing athermic, nondestructive photobiological processes.[5,6] Karu pointed out that irradiation with red light might cause activation of NADPH oxidase because the semmiquinone form of flavin chromophores of this enzyme can absorb light at a wavelength of 632.8 nm.[7] Karu had also suggested that irradiation at dosages from 100 J/m^2 to 300 J/m^2 can significantly induce the respiratory burst of phagocytic cells.[5] Duan *et al.* found protein tyrosine kinases could be crucial in PMN respiratory burst induced by low intensity He-Ne laser irradiation at 300 J/m^2 for 100 seconds.[8] However, low-intensity infrared laser irradiation was found to inhibit the activity of oxidase of phagocytic cells in an intestinal anastomosis.[9] These findings suggested that low-intensity laser irradiation has different cellular effects and might produce opposite PBM at different dosages.[10]

Different results of PBM on PMNs may be related to both PMN amount and its function. Sinyakov *et al.* attempted to evaluate the potential protective capacity of PMNs, and to describe pathology in terms of PMN counts and intra-PMN ROS concentrations.[11] We focused on the function of PMNs and prescind our attention from PMN amount. In this work, we examined the effects of PBM on human PMNs of individual differences due to some factors such as smoking and alcohol that influenced the PMNs' status and PBM on PMNs, and the effects of PBM on PMNs from the athletes at 5 different running stages, and found the key to the different regulatory effects is the status of PMNs themselves and the light dosage.

2. Materials and Methods

2.1. *Polymorphonuclear neutrophil isolation*

Peripheral blood PMNs, from 13 volunteers (10 ordinary persons, 3 athletes), were isolated and purified according to literature described.[12] Blood samples of athletes were extracted at different time period in the 10 km non-interrupted long-distance running: before running, after running began for 1 hour, just finishing the running, resting for 1 hour and 2 hours after running. Cellular viability and purity were checked by trypan blue exclusion and Wright's staining, respectively. Purified PMNs were re-suspended in Hank's balanced salt solution (HBSS) to the required concentration (2×10^6 cells/ml). The proportion of PMNs in the cell preparations needs to be more than 95% and the livability should be more than 98%.

2.2. *Preconditioning of polymorphonuclear neutrophils and irradiation*

Nitroblue tetrazolium (NBT) method can test the activity of NADPH oxidase to assess the intracellular O^{2-} production.[13] We can use this method to check the intensity of the respiratory burst of human PMNs stimulated by the red light (640 ± 15 nm) from light emitting diodes (RLED). One hundred microliter/well of PMN suspension was added into a 96-well microplate for 6 groups with 6 samples in each group. Thirty microliter of NBT was added into each well and then incubated at 37°C for 10 minutes before the irradiation. Five of those groups are for irradiation and the rest are treated as control without irradiation. The output power of RLED is distinguished by the number of the diodes, regulated by diaphragm-mediated system and measured by a power meter. The five dosages were $50\,J/m^2$, $100\,J/m^2$, $300\,J/m^2$, $500\,J/m^2$, $1000\,J/m^2$ and no irradiation for control.[8] The irradiation time was 100 seconds. After irradiation, the suspension was incubated at 37°C again for 30 minutes. One hundred microliter 1 mol/L HCL was added to stop biochemistrial reaction immediately after the incubation. After being centrifugated for 30 minutes at 5000 rpm, the supernate was dropped and HCL was washed out of samples by phosphate-buffered saline (PBS) and then the samples were centrifugated again and this step can be repeated for 2 to 3 times. Then the samples were vaporized in the oven. After that, $200\,\mu l$ dissolution (DMSO (Dimethyl Sulforide): 1 mol/L KOH = 7 : 8) was added into each well, and an oscillator was used to help them dissolve sufficiently. Next, the samples were measured at 490 nm and 570 nm in an absorbance microplate reader.[13]

2.3. *Statistical analysis*

Data are represented as mean ± SEM (standard error of measurement). Statistical analysis was performed by t-test using the SPSS statistical software. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. *PBM on respiratory burst of polymorphonuclear neutrophils from ordinary volunteers*

The PMN suspension samples were irradiated at doses range from $50\,J/m^2$ to $1000\,J/m^2$. Different dosages of RLED have different effects on respiratory burst of PMNs from different groups: three of them, the inducing group, were induced by irradiation in Fig. 1; two of them, the inhibiting group, were inhibited in Fig. 2; and the rest of them, the no-effect group, showed no significant changes in Fig. 3. There are individual differences, but in the same effect group, the general trend is nearly the same.

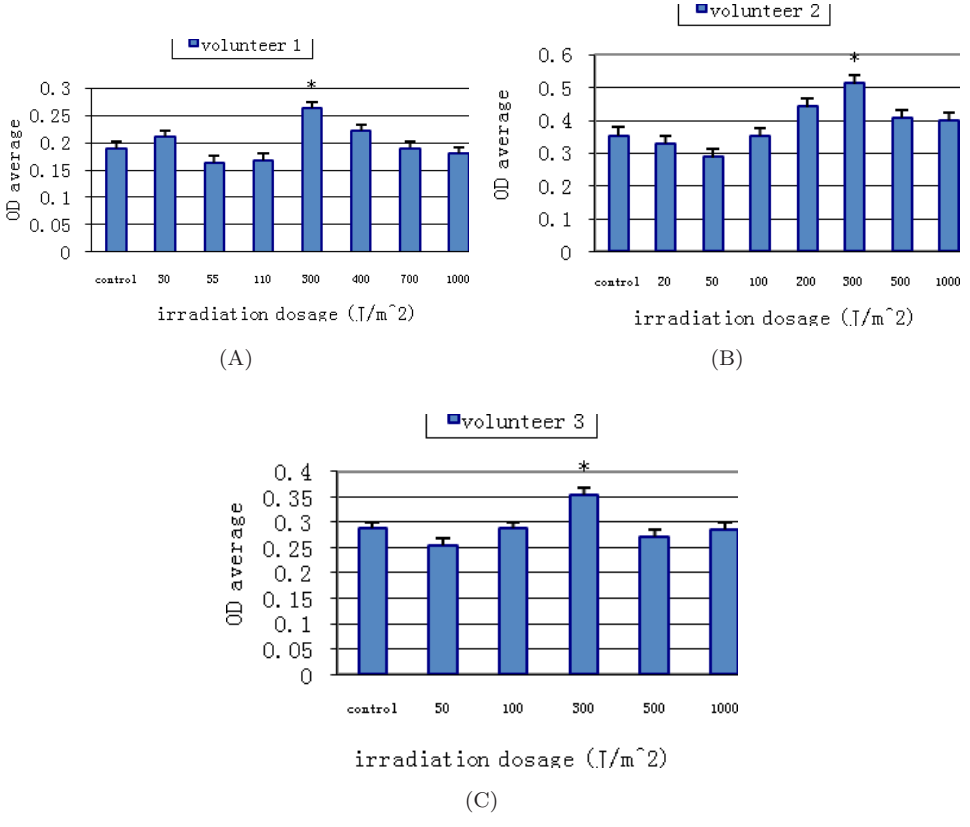

Fig. 1. Inducing PBM on ordinary volunteers: RLED at 300 J/m^2 and 100 seconds induced the respiratory burst of PMNs of volunteers 1 to 3 (*means $p < 0.05$). ((A) volunteer 1 has a habit of smoking and drinking tea; (B) volunteer 2 suffers from fatigue; (C) volunteer 3 has a habit of smoking, drinking and eating spicy food.) Optical Density (OD): An expression of the transmittance of an optical element.

3.2. *PBM on respiratory burst of polymorphonuclear neutrophils from athletes*

The irradiation dosages were the same as those for ordinary volunteers, but each athlete provided blood samples at 5 different stages as stated in *Materials and Method*. The results showed that RELD can significantly induce the respiratory burst of PMNs for the former three stages ($p < 0.05$), while significantly inhibit the respiratory burst of PMNs for the latter two stages ($p < 0.05$). It was also found that the PMNs of althletes have a wider responding dose range to the irradiation stimulation than those of ordinary volunteers, from 50 J/m^2 to 1000 J/m^2, and all may affect the respiratory burst of PMNs and are dosage-dependent. The effects of different dosages at the same stage were distinguished significantly, especially at pre-exercise stage and there were significant individual differences among the 3 athletes, which are illustrated in Figs. 4 to 6.

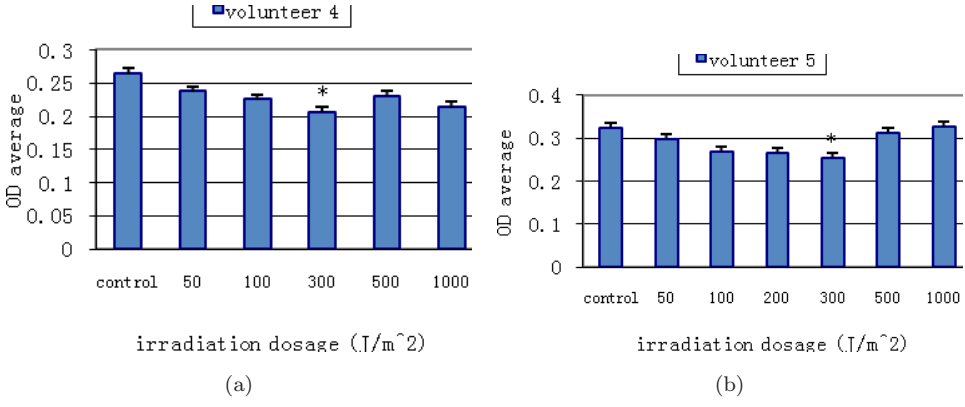

Fig. 2. Inhibiting PBM on ordinary volunteers: RLED at 300 J/m^2 and 100 seconds suppressed the respiratory burst of PMNs of volunteers 4 and 5 (*means $p < 0.05$) (A: volunteer 4 suffered from chronic bronchitis; B: volunteer 5 caught a cold and liked eating spicy food).

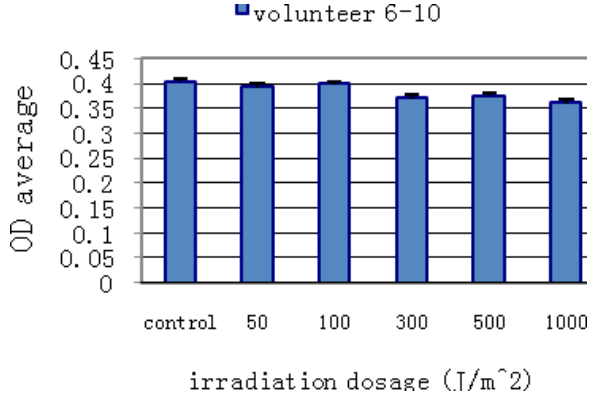

Fig. 3. No significant PBM on ordinary volunteers: RLED at all doses have no significant effects on the respiratory burst of PMN of volunteers 6–10.

3.3. *Discussion*

Although PMNs are essential to host defense, they are also related to the pathology of many chronic inflammatory conditions and ischemia-reperfusion injury, so they are double-edged swords.[14,15] How to keep a balance between host defense and host tissue damage related to PMNs is a great challenge to us. A large number of mediators have been reported to modulate PMN functions *in vitro* or *in vivo*.[16–22] The PMNs of volunteers who have habits of smoking, drinking tea and eating spicy foods or who catch a cold or have other diseases might be changed in their status and functions. The PMNs of athletes who undergo high-intensity exercises are also influenced by exercise. In this study, we found that there might be RLED PBM in dysfunctional human PMNs while none in homeostasis. These phenomena were in agreement with the cellular rehabilitation of PBM.[23]

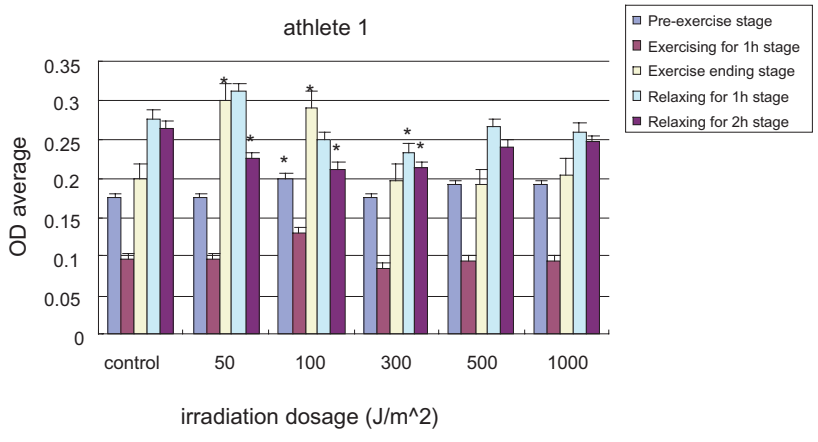

Fig. 4. PBM on athlete 1: RLED induced respiratory burst of PMNs at the former 3 stages and *means OD average at this dosage is significantly different from its control at each stage ($p < 0.05$). RLED inhibited respiratory burst of PMNs at the latter 2 stages and *means OD average at this dosage is significantly different from its control at each stage ($p < 0.05$).

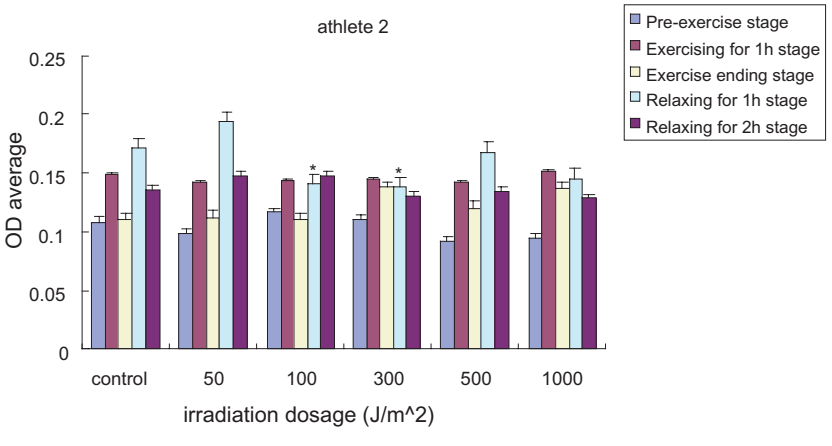

Fig. 5. PBM on athlete 2: RLED induced respiratory burst of PMNs at the former 3 stages and *means OD average at this dosage is significantly different from its control at each stage ($p < 0.05$). RLED inhibited respiratory burst of PMNs at the latter 2 stages and *means OD average at this dosage is significantly different from its control at each stage ($p < 0.05$).

We found that RLED may either have or have no PBM effect on the respiratory burst of PMNs from ordinary volunteers of different health status. In Fig. 3, there were no significant changes of the respiratory burst of PMNs on volunteers 6 to 10 after RLED at all dosages. These volunteers were in good health conditions, and their PMNs were in homeostasis. The homeostasis is the tendency to maintain a constant internal state in response to varying external conditions. Living organisms are characterized by the quality of homeostasis, the ability to self-stablize from internal and external disturbances. A person in good health is generally in homeostasis so

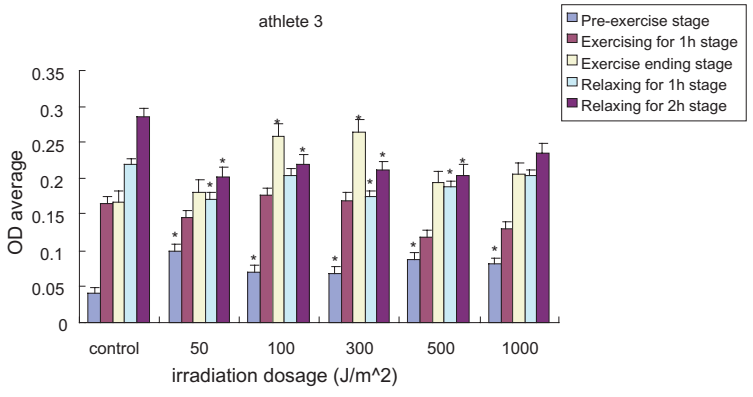

Fig. 6. PBM on athlete 3: RLED induced respiratory burst of PMNs at the former 3 stages and *means OD average at this dosage is significantly different from its control at each stage ($p < 0.05$). RLED inhibited respiratory burst of PMNs at the latter 2 stages and *means OD average at this dosage is significantly different from its control at each stage ($p < 0.05$).

that his neutrophils are, of course, in homeostasis. The PBM is so weak that it cannot disturb the normal respiration burst of their PMNs in homeostasis.

Aside from this light-resistant groups, RLED had significant PBM on the respiratory burst of PMNs of the rest of the ordinary volunteers. These light-responders could be divided into two groups, namely, the inducing group and the inhibiting group. In Fig. 1, RLED showed inducing PBM on PMNs from volunteers 1 to 3, and $300\,J/m^2$ and 100 seconds was the best dosage that induced the respiratory burst of PMNs of volunteers 1 to 3. The following bad habits could jeopardize the normal functions of these volunteers' PMNs as their PMNs were subactivated: habits of taking large amounts of polyphenol of green tea, Gingerones, shogaols, capsaicins, alcohol might all affect physiological status of PMNs.[24–27] The PMNs of chronic alcohol-dependent subjects are dysfunctional in oxygen-dependent antibiotic activities, which impairs the abilities of anti-infection and immune response.[28] Cigarette smoke impairs PMN activation by aldehyde-induced thiol modifications.[29] In these cases, the PMNs are far from homeostasis, but RLED can rehabilitate these dysfunctional PMNs back to homeostasis.

In Fig. 2, RLED showed inhibiting PBM on PMNs from volunteers 4 and 5 and $300\,J/m^2$ and 100 seconds was the best dosage that suppressed the respiratory burst of PMNs from volunteers 4 and 5. In the pathology of many chronic inflammatory conditions, PMNs were over-activated and damaged the host tissues instead of their original protective capacity.[14] In principle, RLED may modulate PMN activation. However, the PMN activation of RLED PBM is weaker than those of inflammatory factors so that RLED cannot promote PMN activitation but may inhibit it instead.

Samples from athletes were studied to confirm the above findings in ordinary volunteers. Each athlete provided blood samples from 5 different stages as stated in *Materials and Method*. Results showed that RLED could significantly induce the respiratory burst of PMNs from the former 3 stages ($p < 0.05$), while significantly

inhibit the respiratory burst of PMNs from the latter 2 stages ($p < 0.05$). A large amount of evidences also indicated that exercise of different intensity can influence the physiological status of PMNs. Intensive exercises seem to inhibit function of immune system and reduce the anti-infective ability.[30–32] Thus, PMNs from the former 3 stages may be in a low level of functions caused by exercises. RLED could rehabilitate these dysfunctional PMNs back to homeostasis and induce PMN respiration burst at several dosages from athletes just like the situation of volunteers 1 to 3. In the latter 2 stages, the PMNs may be over-activated due to microdamage of skeletal muscle. RLED can appease PMNs and rehabilitate them back to homeostasis, and inhibit PMN respiratory burst just like the situation of volunteers 4 and 5.

Sinyakov *et al.* applied leukocyte responsiveness to non-laser blue light to discriminate between pathological and nonpathological states and prognostic evaluation of pathological development.[11] However, Swartz *et al.* identified a functional role for a new type of blue light sensor in bacteria – light, oxygen, or voltage (LOV) histidine kinase.[33] The LOV domain is sensitive to blue light through a noncovalently bound flavin cofactor. In Brucella abortus, blue light increases the enzymatic activity of this kinase, which, remarkably, increases virulence of the bacterium. Thus, more attention should be paid when using blue light in the treatment of PMN-related diseases. Therefore, RLED was chosen to study PBM on PMNs in this paper.

Endonasal low intensity laser therapy (ELILT) began in China in 1998. Now, in China, it is widely applied to treat hyperlipidemia and brain diseases such as Alzheimer's disease, Parkinson's disease, insomnia, poststroke depression, intractable headache, ache in head or face, cerebral thrombosis, acute ischemic cerebrovascular disease, migraine, brain lesion and mild cognitive impairment. Blood was one of the pathways mediated ELILT.[34] Our study implied that ELILT may rehabilitate dysfunctional PMN and the innate natural immune, which would extend the thrapeutic effects of ELILT.

4. Conclusion

There could be RLED PBM on dysfunctional human PMNs, inducing those of subactivated PMNs and inhibiting those of overactivated PMNs, while none on those in homeostasis. The PBM on PMNs is dose-dependent and PMN status-dependent. RLED at 300 J/m^2 for 100 seconds may have bi-direction modulation on PMN respiratory burst, inducing subactivated PMNs and inhibiting overactivated PMNs. Our study is important in extending the therapeutic effects of ELILT.

Acknowledgements

This work was supported by National Science Foundation of China (60478048, 60178003 and 69778003), the Opening Project of MOE Key Laboratory of Laser Life Science, South China Normal University, Guangzhou 510631, China.

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