## Research Article

### Aquaporin-1-Mediated Effects of Low Level He-Ne Laser Irradiation on Human Erythrocytes

Gang-Yue Luo,1,2,3,4 Li Sun,5 and Timon Cheng-Yi Liu1,2,3

1 Laboratory of Laser Sports Medicine, South China Normal University, Guangzhou 510631, China
2 School of Life Science, South China Normal University, Guangzhou 510631, China
3 The Key Laboratory of Ministry of Education of China in Laser Life Science, South China Normal University, Guangzhou 510631, China
4 School of Information and Optoelectronic Science and Engineering, South China Normal University, Guangzhou 510631, China
5 Medical School, Jinan University, Guangzhou 510632, China

Correspondence should be addressed to Timon Cheng-Yi Liu, liutcy@scnu.edu.cn

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The role of membrane aquaporin-1 (APQ-1) in the photobiomodulation (PBM) on erythrocyte deformability will be studied in this paper with human dehydrated erythrocytes as echinocytic shape alterations lead to decreased cellular deformability. Human dehydrated erythrocytes were irradiated with low intensity He-Ne laser irradiation (LHNL) at 0.9, 1.8, 2.7, and 4.4 mW/cm² for 5, 15, and 30 min, respectively, and APQ-1 inhibitor, 0.2 µmol/L HgCl₂, was used to study the role of APQ-1 in mediating PBM with LHNL at 4.4 mW/cm² for 5 min. Comprehensive morphological parameters of an intact cell such as contact area, perimeter, roundness and erythrocyte elongation index (EEI) were measured to characterize erythrocyte deformability with fast micro multi-channel spectrophotometer. It was observed that the dosage of LHNL improvement of the morphological parameters of dehydrated erythrocytes was morphological-parameter-dependent, but the Bunsen-Roscoe rule did not hold for roundness. The LHNL at 4.4 mW/cm² for 5 min significantly improved the contact area (P < 0.05) and EEI (P < 0.05) of the dehydrated erythrocytes, but the improvement was significantly inhibited by 0.2 µmol/L HgCl₂ (P < 0.05). It was concluded that AQP-1 might mediate the effects of LHNL on erythrocyte deformability, which supports the membranotropic mechanism of PBM.

### 1. Introduction

Photobiomodulation (PBM) is a modulation of laser irradiation, monochromatic light, hot color light such as red, orange, or yellow, or cold color light such as green, blue or violet (LI) on biosystems, which stimulates or inhibits biological functions but does not result in irreducible damage. The LI used in PBM is always low intensity LI (LIL), ∼10 mW/cm². However, moderate intensity LI (MIL), 10²–³ mW/cm², is of PBM if the radiation time is not so long that it damages organelles or cells. The PBM of LIL/MIL (LPBM/MPBM) has been widely used to ameliorate hemorheologic behavior of patients [1], but the mechanism of PBM on red blood cells (RBCs) has not been cleared up. MPBM might be mediated by reactive oxygen species (ROS) [1, 2]. Kujawa et al. found MIL promoted at 200 mW but inhibited at 400 mW Na⁺-K⁺-ATPase activity in isolated RBC membranes [3]. Mi et al. found that the deformability of RBCs from pathological samples and Ca²⁺-treated samples was improved after MIL [4]. They further found that MIL can reduce the hemoglobin (Hb) contents in RBCs, and the 532 nm laser was more efficient at lowering Hb than the 632.8 nm laser, consistent with the absorption spectrum of Hb [5].

However, LPBM mechanisms have not been well understood. It has been suggested that LPBM might be mediated by cytochrome c oxidase in mitochondria [6], which has been supported by Wong-Riley et al. [7]; however, there has been the LPBM on RBCs with no mitochondria. RBC solution samples from healthy volunteers were assigned to three groups: the aliquots in Group 1 were irradiated with LIL within 2 h after sampling, and the aliquots in Group 2

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and Group 3 were stored at 5°C for 24 and 36 h, respectively, and received LIL after 12 h (in both groups), 24 h (in Group 2), and 36 h (in Group 3) from sampling. Iijima et al. found that the deformability was unchanged in Group 1 (fresh cell group) from the control value, but improved significantly in Groups 2 and 3 (damaged cell groups) after the LIL [8].

Spodaryk found no effects of LIL on the erythrocyte elongation index (EEI) of RBCs from healthy volunteers [9]. Ku jawa et al. found LIL promoted Na⁺⁻K⁺-ATPase activity in isolated RBC membranes [3].

Function-specific homeostasis (FSH) developed from homeostasis is a negative-feedback response of a biosystem to maintain the function-specific conditions inside the biosystem so that the function is perfectly performed [1, 2]. A biosystem in an FSH means the function is in its FSH so that it is perfectly performed. A biosystem far from a FSH means the function is far from its FSH so that it is dysfunctional. The deformability of RBCs from healthy volunteers [4, 9] is in deformability-specific homeostasis (DSH), but the deformability of RBCs from pathological samples [4], Ca²⁺-treated samples [4], or damaged cell groups [8] is far from DSH. As discussed above, LIL has no direct effects on RBC deformability in its DSH, but modulates the deformability far from its DSH. This homeostatic LPBM has been supposed to be mediated by membrane proteins [1, 2]. This membranotropic hypothesis has been supported by the LPBM on Na⁺⁻K⁺-ATPase activity in isolated RBC membranes [3]. In order to test the membranotropic hypothesis, the role of the membrane aquaporin-1 (APQ-1) in human RBCs in LPBM on RBC deformability far from its DSH was studied in this paper.

2. Materials and Methods

2.1. Blood Samples. Fresh blood from young healthy donors was immediately treated with anticoagulant heparin in tubes. Erythrocytes were collected in the bottom part of the tube after separated from the blood by centrifuging at 1200 xg for 5 min and moving out the blood plasma in the upper part of the centrifuging tube. Some of the erythrocytes were taken out and suspended in hypertonic solution with 5 mmol/L glucose inside at 5% hematocrit, and the others were washed out with hypertonic solution (138 mmol/L NaCl, 5 mmol/L KCl, 10 mmol/L HEPES, and pH 7.35). It was done three times with centrifuging at 1000 xg for 10 min each time. The dehydrated erythrocytes were suspended in the hypertonic solution with 5 mmol/L glucose inside at 5% hematocrit.

2.2. Laser Irradiation. Low intensity He-Ne laser irradiation (LHNL) with 632.8 nm wavelength, 0–5 mW adjustable and continuous power output, and 12 mm beam diameter (MODEL 500-C, Guangzhou Research Institute of Laser Technology, China) were used. LHNL was applied vertically with the powers of 1.0, 2.0, 3.0 and 5.0 mW (intensities: 0.9, 1.8, 2.7, and 4.4 mW/cm²), and radiation times of 5, 15, and 30 min, respectively.

2.3. Measurements of Morphological Parameters and Erythrocyte Elongation Index. After stereo and phase-contrast images of living RBCs were observed by a multidimensional microscope [10], their morphological parameters such as contact area, perimeter, and roundness were determined for one hundred erythrocytes [11, 12], and the EEI was calculated [9].
2.4. Erythrocytes Were Dehydrated through AQP-1 When Treated with Hypertonic Solution. To testify if normal erythrocytes were dehydrated through water channel AQP-1 when treated with the hypertonic solution, the specific inhibitor of AQP-1, HgCl₂ [13], was added. Normal erythrocytes were treated with HgCl₂ (0.2 µmol/L) [13] and then with the hypertonic solution.

2.5. Aquaporin-1 Mediated the Effects of Laser Irradiation. The dehydrated erythrocytes were treated with 0.2 µmol/L HgCl₂ [13] the specific blockers of AQP-1, and incubated in the hypertonic solution for 10 min at 25°C, then irradiated with LHNL at 5 mW for 5 min.

2.6. Statistical Analysis. Data are expressed as means ± standard (SD). The statistical significance was evaluated by covariance analysis and by Student’s t-test with software SPSS 13.0.

3. Results

3.1. Effects of Laser Irradiation on the Morphology of Erythrocytes. The erythrocyte contact area and perimeter are positively relative with cell volume. As shown in Figures 1 and 2, the contact areas and perimeter were increased by the LHNL at 1.0, 2.0, 3.0, and 5.0 mW for 15 and 30 min, and at 3 and 5 mW for 5 min, respectively (P < 0.05). Among them, the best radiation time was 30 min for LHNL at 1.0 mW, 30 min for LHNL at 2.0 mW, 15 min for LHNL at 3.0 mW, and 5 min for the LHNL at 5.0 mW.

The roundness represents the shape of erythrocytes. The more the roundness is close to 1, the more the cell is likely to be spherical. As shown in Figure 3, LHNL increased the roundness at 1 and 2 mW for 15 and 30 min, respectively (P < 0.05), but has no effects at 3 and 5 mW. Among them, the best radiation time was 15 min for LHNL at 1 and 2 mW, respectively.

EEI indicates the deformability of erythrocytes. As shown in Figure 4, LHNL increased EEI only at 5 mW for 5 min (P < 0.05).

3.2. HgCl₂ Inhibited the Effects of Hypertonic Solution on the Deformability of Erythrocytes. HgCl₂ is a specific inhibitor
of AQP-1 [13]. In Figure 5, hypertonic solution decreased erythrocyte contact area \((P < 0.05)\), but failed to do it when treated with 0.2 \(\mu\)mol/l HgCl\(_2\).

As Figures 1–4, and previous paper [8] have shown, LHNL may improve the RBC deformability far from its DSH. In Figure 6, LHNL at 5 mW for 5 min increased the contact area and EEI of dehydrated erythrocytes \((P < 0.05)\), but failed to do it when treated with 0.2 \(\mu\)mol/l HgCl\(_2\).

4. Discussion

The present study demonstrated that LHNL may improve the deformability of human RBCs in the hypertonic solution, and the improvement might be mediated by membrane APQ-1. These results supported the membranotropic hypothesis of LPBM.

The LPBM on erythrocyte deformability has been found to be homeostatic. By using filter filtration rate, Iijima et al. found LHNL improved the erythrocyte deformability far from its DSH, but had no effects on erythrocyte deformability in its DSH [8]. By using EEI, Spodaryk found no LPBM on erythrocyte deformability in its DSH [9]. The erythrocyte deformability was induced far from its DSH in hypertonic solution in this study. By using contact area, perimeter, roundness, and EEI, we found the dosage of LPBM was morphological parameter dependent.

The reciprocity rule, Bunsen-Roscoe rule [6], should not hold, and LPBM should depend on intensity or radiation time if the dose is kept constant. From the observations of different research groups and their own observations, Sommer et al. concluded that the threshold parameters dose and intensity are biologically independent from each other [14]. Lanza
tame et al. have studied the effects of red light at 670 nm from light emitting diode array on pressure ulcers of C57/BL mice, and found varying irradiance and exposure time to achieve a specified energy density affects phototherapy outcomes [15]. The reciprocity rule does not hold in our experiment in Figure 3. The two protocols \((5 \text{ min} \times 3 \text{ mW and } 15 \text{ min} \times 1 \text{ mW})\) gave the same dosage of LIL, but induced different changes in the roundness of erythrocyte \((P < 0.05)\), the former one \((5 \text{ min} \times 3 \text{ mW})\) caused no significant difference from the control group, but the later one \((15 \text{ min} \times 1 \text{ mW})\) has significant differences from control group.

Normal erythrocytes contract and expand reversibly when hypertonic and hypotonic solution are added, but the deformability was far from its DSH when these two solutions were added [16] and even erythrocytolyis can be caused [17]. Specific water channel AQP-1 in erythrocyte membrane can mediate fast and active transport of water molecular through membranes. In numerous physiology responses, this transfer of water molecular through erythrocyte membrane by AQP-1 can have a lasting effect and cause the change of endoplasm viscosity [18]. HgCl\(_2\), an inhibitor of AQP-1, can inhibit the transportation mentioned above [13]. As Figure 5 has shown, normal erythrocytes become small in hypertonic solution, but HgCl\(_2\)-pretreated normal erythrocytes do not. It is worth noting that PBM can inhibit erythrocytolyis in hypotonic solution [16]. In this study, by using multiparameter dynamic measurement of elastic
properties of human erythrocyte and water channel AQP-1 inhibitor HgCl₂, we investigated the mechanism of modulation of dehydrated erythrocyte morphological character by LHNL, and found that HgCl₂ can inhibit the LPBM of dehydrated erythrocyte. As Figure 6 has shown, dehydrated erythrocytes become large after LHNL, but HgCl₂-pretreated dehydrated erythrocytes do not. This suggests that the LPBM of dehydrated erythrocyte morphological might be mediated by AQP-1.

5. Conclusion
AQP-1 might mediate the effect of LHNL on human erythrocyte deformability, which supports the membranotropic mechanism of LPBM.

Authors’ Contribution
G.-y. Luo and L. Sun are contributed equally to this work.

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