Mechanism of low power laser irradiation on red blood cell deformability

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The effects of laser irradiation on erythrocyte deformability and related mechanism were investigated. Membrane attached hemoglobin (Hbm) is the determinant factor of erythrocyte deformability. After irradiation of 532 nm (30 mW, 20 minutes) or 632.8 nm (30 mW, 60 minutes), the content of Hbm lowered and the deformability of erythrocytes increased, indicating that the decreasing of Hbm is one of the mechanisms of laser action to improve the erythrocyte deformability. The 532-nm laser is more efficient than 632.8 nm laser in the action, which is consistent with the absorption spectrum of Hbm, confirming that the Hbm is the target molecule under laser irradiation.

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During the past two decades, the effects of low power laser irradiation were extensively studied[1,2] and a large number of clinical studies with low power laser irradiation have been used to treat various pathologic processes[3,4]. In China, the intravenous laser irradiation has been applied clinically to treat various diseases such as acute cerebral infarction, and the results have been encouraging[5,6]. It is deduced that the blood microcirculation in brain may be improved by laser irradiation, which promotes the recovery of patients.

In previous study, we have found laser irradiation not only improved blood viscosities at low shear rates but also at high shear rates[7]. Since the erythrocyte deformability is closely correlated with blood viscosity at high shear rates[8], it is suspected that low power laser irradiation may increase the erythrocyte deformability. In this work, we intend to explore the effect of low power laser irradiation on erythrocyte deformability and related mechanism. The He-Ne laser (632.8 nm) and YAG laser (532 nm) were used in the experiments. Under laser irradiation (30 mW on a spot with a 5-mm diameter), no hemolysis or morphological changes of the erythrocytes were observed.

Table 1. Effect of Laser Irradiation on Erythrocyte Deformability

	Reference	632.8 nm 5.4 J/mm^2	650 nm 5.4 J/mm^2	532 nm 1.8 J/mm^2
Abnormal Samples (n=13)				
IF	0.225	0.202	0.199	0.177
	±0.008	±0.006	±0.038	±0.008
p Value		< 0.05	< 0.01	< 0.01
Mean RV		9.32	11.56	19.95
(%)		±3.13	±2.79	±6.76
Normal Samples (n=6)				
IF	0.148	0.159	0.163	0.164
	±0.007	±0.003	±0.012	±0.007
p Value		> 0.05	> 0.05	> 0.05

n: number of samples; RV: relative variation.

The deformability of normal and pathological samples of erythrocytes were measured by the nucleopore membrane red cell deformability meter based on pore filtration (DXC-400, Instrumental Factory of Shanghai Medical University) with a repeatability fluctuation less than 3%[9]. The index of filtration (IF) was used to express the relative deformability of the erythrocyte samples[10].

The erythrocyte samples were obtained from 19 patients, in which 13 samples were in abnormal poor deformability (IF> 0.175) and the other six samples were within normal ranges (IF: 0.120–0.175). Each erythrocyte sample was divided into four tubes for referenc, irradiation with 632.8 nm, 650 nm (semiconductor laser), and irradiation with 532 nm. Table 1 shows the results of laser irradiation on erythrocyte deformability. For the normal group (six samples), laser irradiation had no meaningful effect on the change of deformability compared with the referenc samples ($p > 0.05$), no matter which laser was employed (632.8, 650, or 532 nm). For the abnormal group, all the lasers used enhanced deformability of erythrocytes evidently($p < 0.05$ or 0.01). Among the three lasers, the 532-nm laser showed a better effect on improving the erythrocyte deformability.

Incubating the erythrocytes with calcium ions is a common way to obtain erythrocyte samples with poor deformability[11]. To explore the effect of laser irradiation on erythrocyte deformability further, the normal blood samples from healthy people treated with $CaCl_2$ were used to obtain the typical samples with the poor deformability. The treatment was in the following way: after separated from the whole blood, the erythrocyte samples with 5% hematocrit were incubated for 3 hours at 37 °C in an isotonic HEPES buffer (10-mmol/L HEPES, 150-mmol/L NaCl, 5-mmol/L KCl, PH=7.4, 290 mOsm/kg) containing 0 and 1.25-mmol/L of isotonic $CaCl_2$ solution. Five samples were prepared at each condition for experiments. Each sample was then divided into three tubes for irradiation (632.8 and 532 nm) and non-irradiated reference. Then the erythrocyte deformability was measured.

The results demonstrate that irradiation of both 632.8- and 532-nm lasers remarkably reduced the rigidity of $CaCl_2$-treated erythrocytes (see Table 2), confirming

Table 2. Effect of Laser Irradiation on Erythrocyte Deformability ($CaCl_2$ Treated Samples)

$CaCl_2$ (mmol/L)	IF of Reference	IF after Irradiation of 632.8 nm, 5.4 J/mm^2	Mean RV (%)	IF after Irradiation of 532 nm(1.8 J/mm^2)	Mean RV (%)
0 (n=7)	0.251±0.022	0.229±0.022**	8.49±1.77	0.206±0.024**	12.92±3.09
1.25 (n=4)	0.547±0.013	0.456±0.034*	16.65±6.03	0.412±0.032**	24.71±5.48

*$p < 0.05$; **$p < 0.01$.

the laser effect on improving deformability of erythrocytes. Here a similar trend was also observed that 532-nm laser is more effective than 632.8-nm laser. The relative variation of erythrocyte deformability is obviously higher in 532-nm irradiated samples than that in 632.8 nm irradiated samples. Comparing the results of laser irradiation with 532 and 632.8 nm, the wavelength effect is obvious; i.e. that the laser of 532 nm functioned better than the laser of 632.8 nm did at the same conditions.

Why $CaCl_2$ incubation lowered deformability of erythrocytes and laser irradiation can improve the erythrocyte deformability in these $CaCl_2$ treated erythrocytes? As we know, the membrane-attached hemoglobin (Hbm) is one of the determinant factors of erythrocyte deformability. There are good correlations between Hbm and average erythrocyte rigidity, that more Hbm molecules the erythrocyte contains, the worse its deformability is[12,13]. The common binding site of Hbm is the band 3 protein of erythrocyte membrane[14]. When the laser beam irradiates the erythrocytes, the Hbm molecules on the membrane could absorb the incident photons and then disconnect from the erythrocyte membrane. Such a hypothesis seems reasonable. Here, the experiment of erythrocyte ghost treated by $CaCl_2$ was designed to convince it. Because the response of pig's erythrocytes to laser irradiation was similar with that of human erythrocytes[15,16], the pig's erythrocytes were used in this experiment.

The erythrocyte ghost were prepared by adding 1 ml of ice-cold erythrocyte suspension (50% hematocrit) to 3 ml of 0 °C, hypotonic HEPES buffer (20-mmol/L HEPES, 5-mmol/L KCl, PH 7.4, 40 mOsm/kg) containing different concentration of $CaCl_2$ in different sample group. According to the previous report, $CaCl_2$ makes Hb attach on erythrocyte membrane[12]. Here the $CaCl_2$ concentrations of 0, 0.5, 1, and 2 mmol/L were added in each sample buffer respectively. After 40 minutes incubation, each sample was centrifuge washed three times (20000 g for 10 minutes) with pure water, and the erythrocyte ghosts in each sample were collected and re-suspended in pure water. In these ghosts the additional Hbm might be induced. Then each ghost suspension was divided into two tubes for laser irradiation and reference. After irradiation, all the samples including irradiated and un-irradiated groups were centrifuge washed again to remove un-bound Hb molecules. Finally the Hbm content of all samples (final ghost suspensions) was measured through UV-visible Spectrometer (V550, JASCO Corporation, Japan). The absorption spectra as well as its absorption values of the samples at 415 nm ($\Delta\lambda = 5$ nm) were obtained from the measurements.

Here, the same effect of $CaCl_2$ was found in pig's erythrocyte ghosts, the changes of Hbm content were seen from their absorption spectra, as shown typically in Fig. 1. The OD value of Hbm with the incubation of $CaCl_2$ (1 mmol/L) is 0.55 (before irradiation), and decreases to 0.47 with 14% relative changs after irradiation of 532-nm laser (1.8 J/mm^2). The laser effects on lowering the Hbm content are shown in Table 3 in detail, that both lasers (532 and 632.8 nm) have obvious effect statistically, but 532 nm laser is more efficient than 632.8-nm laser.

It is noticed that when the Hbm is in original level (OD about 0.24), no effects can be found after laser irradiation. These phenomena could be understood as that the erythrocytes have their normal Hbm level, at which level the erythrocytes have their normal deformability. While the Hbm is in abnormal higher level, the deformability of

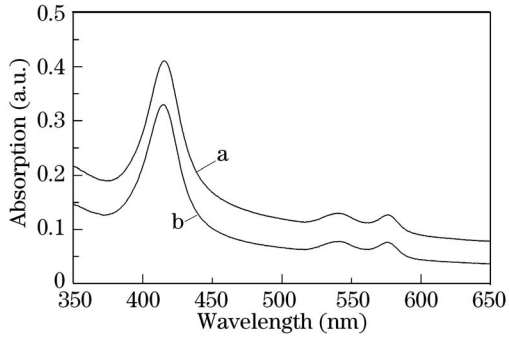

Fig. 1. Absorption spectrum of Hbm. Curve a: the erythrocytes were treated with $CaCl_2$ (1 mmol/L) before laser irradiation; curve b: after of 532-nm, 1.8-J/mm^2 laser irradiation.

Table 3. Effects of Laser Irradiation on Hbm of Erythrocytes Treated by $CaCl_2$

$CaCl_2$ (mmol/L)	Reference	Hbm (OD Value) Irradiation	Mean RV (%)	p Value
		532 nm, 1.8 J/mm^2		
0	0.243±0.020	0.241±0.021		> 0.05
2	0.553±0.038	0.474±0.035	14.28±1.78	< 0.01
		632.8 nm, 1.8 J/mm^2		
0	0.240±0.020	0.245±0.021		> 0.05
2	0.520±0.038	0.521±0.042		> 0.05
		632.8 nm, 5.4 J/mm^2		
0	0.242±0.035	0.244±0.038		> 0.05
2	0.517±0.033	0.440±0.027	14.79±5.50	< 0.05

Data are mean±SD (standard deviation) from 5 experiments.

erythrocytes becomes worse[12]. Here, being treated with 2-mmol/L $CaCl_2$, the erythrocyte ghosts had abnormal Hbm level (OD about 0.5). After laser irradiation (532 nm at 1.8 J/cm^2 or 632.8 nm at 5.4 J/cm^2), the 14 % reduction of Hbm content could be obtained.

As considered from the beginning of this report, why does laser irradiation improve microcirculation? The results of this work may give an answer. The improvement of erythrocyte deformability is therefore the possible cause of the improvement of the microcirculation. Under laser irradiation, the Hbm is the target molecule. Laser irradiation reduces Hbm content of erythrocytes, which is one of the mechanisms of the improvement of erythrocyte deformability.

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