In Vitro Impact of Laser Irradiation on Platelets Aggregation

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Abstract
The review of blood optical and rheological parameters played an important role in many medical routine diagnosis and therapeutic applications, and it is the best design to understand the mechanism of action of the low level laser irradiation on biological tissues. The insistence of this study was to investigate the in vitro effect of laser radiation on platelets number and aggregation. The blood samples were conquered from 30 healthy volunteers, each sample was parted into four aliquots one of them was considered as control while the other three were exposed to three different laser doses. The wavelength of 532 nm was used for irradiation with 4 mm diameter beam spot. The irradiation times were 1.8, 3.7 and 6.2 sec giving different doses of irradiation 1.5, 3 and 5 J/cm², respectively. Low laser irradiations induce significant changes in platelets aggregation in presence of weak agonist as ADP (P≤0.05) and epinephrine (P≤0.01). Low-level laser therapy has no influence on platelets count; however, its promote platelets aggregation in response to weak agonist, specifically ADP and epinephrine.

1 Introduction
Lasers, as highly stable sources of coherent and monochromatic light, have been used extensively in technical applications and for medical therapy. The effect of laser irradiation on biological objects depends on experimental conditions, such as the type of cells irradiated, wavelength and intensity of light, etc. High energy laser irradiation causes destruction and vaporization of tissues, which has been exploited in surgery. It has been shown that low-energy laser light can stimulate the mitosis and protein synthesis in cells, while the cells can be damaged or destroyed when high-energy laser light is applied.

Blood platelets form an interesting biological system whose morphology is sensitive to its environment and any change in this morphology may be critical to the physiology of living organisms. A study of the blood optical and rheological parameters played an important role in many medical routine diagnosis and therapeutic application. When persons are irradiated with low level laser irradiation then it causes several clinical and biological changes namely anti-inflammatory, immune stimulatory, neurotrophic, analgesic, desensitizing, bactericidal, normalizing the blood rheology and hemodynamic effects (depending on the condition of the patient and the pathology). Accordingly, low level laser treatment can be applied for the therapy of several pathologic conditions in various branches of medicine, including disorders.

The physiochemical properties of blood cells changed during treatment with low level laser therapy. Blood platelets imparts chief role in maintaining good health, but platelets are very sensitive and leads to activation (platelet degranulation, procoagulant surface formation, platelet aggregation etc) on exposure to various stimuli. Consequently, it is urgent needs to study the effect of laser light irradiation on blood platelets on different parameters. There is a number of studies dealing with the effect of UV, red and rarely green laser light irradiation on blood platelet damage, activation , adhesion, aggregation and response to various platelet activators following such an irradiation although the outcomes of these studies are conflicting it seems evident that laser light irradiation has a significant effect on platelet physiology, concerning the alterations in their reactivity and consequently
affecting their aggregation and adhesion\(^1\). We planned to study the effect of low level laser radiation on platelets number and aggregation.

2 Materials and methods

2.1 Blood sample

Thirty apparently healthy individuals (18 males and 12 females, mean age 26.97 ± 5.01) were enrolled in this study, with no history of chief illnesses or regular medications especially blood thinner agents. They were instructed about the purpose of the study and verbal permission was taken from all participants. About 10 ml of blood were collected through venipuncture in ethylene diamine tetra acetic acid (EDTA)-containing tubes (1.3 mg/ml of blood) as an anticoagulant. The samples were then managed instantly after collection. Each sample was apportioned into four equal aliquots, one to be used as non-irradiated sample (control) and the other three were exposed to different laser doses using a continuous ND: YAG laser.

2.2 Laser specification

In this study, a diode laser pointer was used as irradiation source with a wavelength of 532 nm, and a low power of 100 mW in a continuous wave mode. The divergence was < 1.5 mRad. The crystal type of this source was Nd: YVO4: KTP. The laser spot diameter was 0.4 cm. The power density was 796.17 mW/cm\(^2\).

2.3 Sample irradiation

The blood samples of 2.5 ml per tube were irradiated with a laser beam of 0.4 cm spot diameter. The delivered dose for each irradiated set was 1.5, 3 and 5 J/cm\(^2\), at different exposure time; 1.8, 3.7 and 6.2 seconds, respectively. The laser beam was focused routinely to the center of the blood containing test tube. The irradiation process was achieved at room temperature (18-25\(^\circ\)C). Total platelets counts (for irradiated and non-irradiated sample) was measured as a part of complete blood picture including (RBC, WBC, Hb, MCV and MCHC) by using a hematology analyzer machine. The platelet aggregation of irradiated and non-irradiated samples was measured automatically by using a Platelet Function Analyzer type (PAP-8E) in response to three different agonist (ADP, epinephrine and arachidonic acid) at the National Center of Hematology (Specialty Center for Blood Diseases)\(^1\)-\(^5\).

2.4 Statistical evaluation

The data is assessed by the use of Excel software; the values are expressed as mean ± standard deviation. The differences between the three irradiated and control samples were estimated by applying a paired \(t\) test. The \(P\) value is determined according to the analysis of the significance of the difference. The \(P\) value less than 0.05 were considered as significant.

3 Results

Table 1 shows the response of blood parameters to different laser doses, of which the MCV and WBC shows significant differences in response to laser irradiation.

3.1 Platelets aggregation response to laser irradiation

The impacts of laser irradiation on platelet aggregation were tested with the aggregating agents, including ADP, Arachidonic acid (AA) and epinephrine. Both the maximum (MA) and final activity (AA) were considered. Since the aggregation test is very expensive, we chose single effective dose depending on the results mentioned in the table 1, which is 3 J/cm\(^2\).

The following changes were observed regarding the ADP and Epinephrine in disparity to AA (arachidonic acid) which shows no significant differences after irradiation for both maximum and final activity (MA & FA). The mean values of platelets aggregation in response to ADP pre-irradiation for both MA and FA were (74.11±11.09 and 72.66±10.86, respectively) which significantly altered post irradiation to (66.77±14.42 and 65.33±14.11 respectively) with \(P \leq 0.05\) (Fig 1). Likewise for the epinephrine response, the mean values of platelets aggregation pre-irradiation for both MA and FA were (65.66±12.17 and 64.22±11.87, respectively) where differ significantly post-irradiation (51.11±15.15 and 49.77±14.59 respectively) with \(P \leq 0.01\) (Fig 2).

4 Discussions

Human platelets are analytically convoluted in both ordinary hemostasis and abnormal conditions such as hemorrhage and thrombosis. These precise cells provide impressively to blood vessel contraction and renewal\(^6\). Additionally, platelets performing together with further cells including, endothelial and white cells or cells of smooth muscle – an important role in inflammation and in the upgrade of atherosclerosis\(^7\).

The primary hemostasis involves of the interaction among the wall of blood vessels and platelets. Shortly after the destruction of the endothelial surface of the blood vessels, platelets are promptly activated and consequently adhesion, release reaction, aggregation and hemostatic plug achieved preventing further blood loss\(^8\). An increased hazard of hemorrhage could be happen when platelets number is declined and/or one of their functions is imperfect. Contrariwise, inappropriate thrombi formation could be due to elevation in platelet count or reactivity. Actually, activated platelets and its consequences of adhesion and aggregation within atherosclerotic lesions is the main pathophysiology of different thromboembolic disease such as cerebrovascular accident and myocardial infarction\(^7\)-\(^9\).

Light transmission aggregometry (LTA) is considered as the golden ordinary platelet function test and is still the best used analysis for the documentation and identification of platelet function imperfections. Platelet rich plasma (PRP) is agitated inside a cuvette positioned between a light source and a sensor.
After adding of a different pane of agonists, for example collagen, ristocetin, thrombin, epinephrine, ADP, and arachidonic acid, the platelets aggregate and light transmission surges. The platelet aggregation design is assumed as a crucial response to an exogenous agonist, followed by a secondary response to the release of dense granule contents. Similarly, when high concentrations of agonists are employed leads to conceal the biphasic response\textsuperscript{10,11}.

Table 1: The response of some hematological parameters to different laser doses compared to control

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-irradiation (control)</th>
<th>1.5 j/cm(^2)</th>
<th>3 j/cm(^2)</th>
<th>5 j/cm(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>4.96±0.41</td>
<td>4.94±0.43</td>
<td>4.93±0.43</td>
<td>4.92±0.38</td>
</tr>
<tr>
<td>MCHC</td>
<td>32.86±1.71</td>
<td>32.91±1.44</td>
<td>32.98±1.53</td>
<td>32.99±1.34</td>
</tr>
<tr>
<td>RDW</td>
<td>11.73±0.61</td>
<td>11.74±0.66</td>
<td>11.73±0.70</td>
<td>11.72±0.65</td>
</tr>
<tr>
<td>Hb</td>
<td>14.06±1.43</td>
<td>13.97±1.37</td>
<td>13.98±1.36</td>
<td>13.97±1.30</td>
</tr>
<tr>
<td>MCV</td>
<td>86.49 ± 4.89</td>
<td>86.28±4.77</td>
<td>86.18±4.80</td>
<td>86.13±4.85</td>
</tr>
<tr>
<td>Plat.count</td>
<td>275.3±6.96</td>
<td>275.4±5.20</td>
<td>276.9±6.90</td>
<td>272.9±7.88</td>
</tr>
<tr>
<td>WBC</td>
<td>7.79± 2.17</td>
<td>7.83± 2.04</td>
<td>7.91±2.13</td>
<td>7.92±2.13</td>
</tr>
</tbody>
</table>

Fig 1: Platelets aggregation response to ADP (pre and post laser irradiation)

Figure2: platelets response to epinephrine (pre and post laser irradiation)

Traditional LTA remains the best beneficial procedure for detecting a wide variety of platelet imperfections. The main weakness of LTA is the use of PRP in place of the whole blood under relatively low shear circumstances, and, in the lack of erythrocytes and leukocytes, it does not accurately mimic primary hemostasis\textsuperscript{12}. Various clinical applications were documented the effectiveness of laser irradiation in correcting thrombocytic dysfunction.

Corresponding, the management and prevention of chronic venous ulcers, with good patient gratification and minor complication\textsuperscript{13}. Recently, Successful ablation was achieved in 100% of veins independent of size using endovenous laser therapy\textsuperscript{14}.

Actually, the He-Ne induced laser carotid artery thrombus model. This model APN-KO mice exhibited an accelerated thrombus formation, platelet aggregometry and the real-time observation of \textit{in vitro} thrombus formation on a type I collagen-coated surface under flow conditions showed the enhanced platelet function\textsuperscript{15-17}.

In this work three types of agonist were tested, including ADP, Arachidonic acid (AA) and epinephrine. The experimental data revealed that, laser irradiation promote platelets aggregation in response to ADP and epinephrine which is in synchronization with previous reports mentioned above. Although this reported in vitro, it should be well-thought-out in vivo.

As laser therapy widely used worldwide for different medical conditions, the risk of the consequences of platelets activation is not surprising and may contribute to relapsing rate and complication sequel. Such finding guarantees with Puggioni et al (2005)\textsuperscript{18} suggested who proposed that, older patients tend to develop more proximal great saphenous vein thrombi after varicose ablation by using endovenous laser therapy therefore prophylactic medication may be considered.

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5 Conclusion

Based on the study analyzed, low-level laser therapy has no influence on platelets count, however its promote platelets aggregation in response to weak agonist specifically ADP and epinephrine. Indeed precaution during laser application is obligatory, particularly in patient at risk of thrombosis and antidote may necessitate.

6 Conflict of interest

None conflict

7 Author’s contributions

EAS carried out complete experimental work and drafted the manuscript. I read and approved the final manuscript.

8 Role of funding source

Personal

9 Ethical approvals

Not required

10 Informed consent

All participants were instructed about the purpose of the study and verbal permission was taken.

11 References


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