

Effects of Q-switched Nd: YAG laser irradiation on the aqueous solution of human albumin and calf-thymus double strand DNA (ctds)

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Abstract

Q-switched lasers widely used in management skin diseases and sometimes its effect may be inadequate or associated with cytotoxicity. The current study aimed to investigate the effect of Q-switched Nd:YAG laser upon cellular elements using in vitro experimental model. Aqueous solutions of human albumin and pure calf thymus double strand deoxyribonucleic acid (ctdsDNA) irradiated with Q-switched Nd:YAG laser at different rates (1, 3 Hz) and time exposure (up to 60 seconds) using 532 nm (400 mJ) and 1064 (1200 mJ) nm wavelength with fixed spot size of 4 mm. The effect of laser irradiation on the albumin solution also studied in the presence of elemental salts of copper, zinc and iron.

Q-switched laser irradiation at 532 nm produced albumin molecule damage that potentiated by copper sulfate and variable effect with zinc sulfate. Iron salt stabilized the effect of laser irradiation upon albumin molecule. The effect of laser irradiation at 1064 nm is less than that observed with 532 nm wavelength. ctdsDNA strands are more susceptible to Q-switched Nd:YAG laser radiation when exposed at 532 nm with pulse rate of 3Hz. It concludes that using Q-switched Nd:YAG laser is safe upon the cellular elements at 1064nm, pulse rate 1 Hz and spot size of 4 mm.

Key words

Q-switched Nd:YAG laser, Albumin, DNA.

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تأثيرات اشعاع الليزر العامل بمفتاح تشغيل النوعية Nd: YAG على المحلول المائي

للألبومين البشري والحمض النووي الرايبوزي منقوص الأوكسجين مزدوج الشريط (DNA)

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الخلاصة

يستخدم الليزر العامل بمفتاح تشغيل النوعية بشكل واسع لعلاج الامراض الجلدية وفي بعض الاحيان يكون غير كاف ومصحوبا بتأثيرات سمية للخلايا. لذلك كان هدف البحث دراسة تأثير الليزر العامل بمفتاح تشغيل النوعية Nd:YAG على مكونات الخلية بتوظيف تجريبي خارج الجسم الحي. تم العمل على تشيع المحلول المائي لألبومين قياسي ودنا مزدوج الشريط للغدة الزعترية للعجل (ctdsDNA) باشعة الليزر العامل بمفتاح تشغيل النوعية Nd:YAG بترددات مختلفة (1، 3 هيرتز) لفترات زمنية متغيرة (0- 60 ثانية) باستخدام طول موجي 532 نانومتر و1064 نانومتر على بقعه حجمها 4ملم كما تم دراسة تأثير أشعة الليزر على محلول الألبومين بوجود املاح عناصر أثرية (النحاس، الزنك والحديد).

استنتج من ذلك أن اشعة الليزر العاملة بمفتاح تشغيل النوعية عند الطول الموجي 532 نانومتر أحدثت ضرر لجزيئي الألبومين، وقد ازداد الضرر بوجود كبريتات النحاس في حين كان التأثير مغاير مع كبريتات الزنك. أما املاح الحديد فقد حافظت على توازن تأثير اشعة الليزر في جزيئي الألبومين. لوحظ أيضا تأثر جزيئي الحمض

النووي الرايبوزي منقوص الأوكسجين مزدوج الشريط (DNA) بالطول الموجي 532 نانومتر لاشعة الليزر العاملة بمفتاح تشغيل النوعية Nd:YAG بمعدل نبض 3 هيرتز. وكذلك أظهرت هذه الدراسة ان تطبيقات الليزر العامل بمفتاح تشغيل النوعية Nd:YAG يكون آمن على مكونات الخلية بالطول الموجي 1064 نانومتر ومعدل نبض 1 هيرتز وبقعه حجمها 4 ملم.

Introduction

Nowadays the skin tattoo are usually removed by Q-switched lasers and sometimes its effect may be inadequate. The effect of removing skin tattoo can be enhance by using a combination of Carbon dioxide laser (CO₂ laser) and Q –Switched Nd:YAG (1064 nm) laser which showed effective removal of blue-black/blue amateur tattoo in pigmented skin [1]. Using Q-switched Nd:YAG laser with a continuously variable spot-size effectively removes tattoos and a greater removal when used a larger spot-size i.e. 4 mm spot[2]. The cytotoxicity of Q-switched ND:YAG laser studied in vitro and in vivo studies. Mice treated with repeated irradiation with 1,320 nm Nd:YAG laser up to six weeks did not cause signs of cytotoxicity in term of mortality, decrease body weight or appearance of skin tumor [3]. Cultured human skin fibroblasts irradiated with the 1.5 J/cm² of 532 nm and 1,064 nm lasers significantly expressed type I and III procollagen, tissue inhibitors of metalloproteinases, and a decrease expression of matrix metalloproteinases[4]. In another study, the dorsal skin of rats irradiated with the 1064 nm laser at different fluencies found marked increase collagen synthesis and inhibit collagen degradation [5]. Some authors [6] attributed the increase in collagen synthesis to the cellular improvement of antioxidants and reduced oxidative stress that stimulate the collagen synthesis. On the other hand, the genotoxic effect of Q-switched ND:YAG laser observed in fibroblast cell culture in term of increase DNA damage in different cell culture

passages as assessed by comet test [7]. The rational of this study is that the laser irradiation disrupted the keratin or corneocytes completely and perforating some micropores on the stratum corneum of the skin [8] which may cause damage to the cell constituents like proteins and DNA. Therefore, this study aimed to investigate the effect of Q-switched ND:YAG laser (532 nm and 1064 nm) on the aqueous solution of human albumin and calf thymus double stands DNA (ctdsDNA).

Materials and methods

This study conducted at Department of Physiology/Medical Physics, College of Medicine, Al-Mustansiriya University from October to December 2013. The project of this study approved by the Institutional Scientific Committee. The experiments were done on the human albumin and pure calf thymus double strand deoxyribonucleic acid (ctdsDNA) of molecular weight 3.56 x10³g/mol, calculated from SV20. An aqueous solution of each human albumin (concentration or ctdNA) exposed to laser beam radiation at different frequency rates (1, 3 Hz) and exposure time (up to 60 seconds). Aqueous human albumin solution prepared freshly using distilled water as a solvent to obtain a final concentration of (50 µg/ml). Aqueous ctdsDNA solution prepared freshly by dissolving known weigh of ctdsDNA in isotonic citric solution (0.0015M sodium chloride, 0.00015 trisodium citrate) to obtain a final concentration of 50µg/ml.

Aqueous albumin solution in glass test tube was exposed to laser beam radiation from a 1cm distance at wavelength 532 nm (pulse rate 1 Hz) (energy 400mJ) and 1064 nm (pulse rate 3 Hz) (energy 1200mJ) for 15, 30, 45 and 60 seconds irradiation, in presence or absence one of the following salts; CuSO₄, ZnSO₄ and FeSO₄ (1 mmol). The concentration of albumin determined spectrophotometrically using Bradford's method [9]. In brief 100µl of aqueous albumin solution (50µg/ml) added to 5 ml of Bradford's solution (prepared by dissolving 100 mgcomossie reagent 250G in 50 ml

ethanol and 100 ml of phosphoric acid (85%) and the volume was completed with distilled water up to 1L). The absorbance of aqueous solution measured spectrophotometrically at 595 nm. The mean of triplicate samples for each treatment as well as non-treated samples served as control was calculated. An increase absorbance magnitude indicated separation of protein peptides whereas a decrease in the absorbance magnitude indicated fragmentation of protein molecule. The effect of laser assessed by calculating the percent changes using the following equation:

$$\% \text{ changes} = \frac{\text{Concentration (after laser -before laser)}}{\text{Concentration before laser}}$$

Aqueous solution ctdsDNA irradiated with laser beam at wavelength 532 nm and 1064 nm at pulse rate 1 and 3 Hz for 10,20,30 and 40 seconds. The absorbance(O.D.) of triplicate samples for each treatment as well as non treated samples served as control were recorded at wavelength 260 nm using UV-visible spectrophotometer. An

increase absorbance magnitude indicated hyperchromnasia i.e. longitudinal separation of DNA strands and a decrease in absorbance magnitude indicated hypochromasia i.e. DNA strands break. The concentration of ctdsDNA after irradiation calculated by using the following equation:

$$\text{ctdsDNA concentration after irradiation} = \frac{\text{Absorbance at 260 nm (after laser)}}{\text{Absorbance at 260 nm (before laser)}} \times \text{concentration of ctdsDNA (50µg/ml)}$$

The effect of laser was assessed by calculating the percent changes as mentioned above.

The specification of laser system included:

- Laser source: Nd:YAG
- Wavelength (1064 nm) energy (1200 mJ), Pulse width Q-switched 1064nm, 10ns.
- wavelength (532 nm) energy (400mJ).
- Operation mode: Q-switched, and free running mode.
- Spot size: adjusted to 4mm.

All chemicals were of analar grade. Human albumin (Audit Diagnostica,

32g/L concentration) generously obtained from the Laboratories of Al-Yarmouk Teaching hospital in Baghdad and ctdsDNA purchased from BDH chemicals, England.

Results

1. Effect of laser radiation on the albumin aqueous solution

Fig. 1 shows that the absorbance of aqueous albumin solution increased after laser irradiation (532 nm, pulse rate 1 Hz) at any interval time of exposure to reach 114% after 30 seconds radiation i.e. 14% increment. Further increment in absorbance

observed when the albumin aqueous solution incubated with CuSO_4 (1mmol) prior to the irradiation to reach 157.5% after 15 seconds radiation i.e. 57.5 % increment. Zinc sulfate salt produced opposite effect i.e. the absorbance reduced to 31.9% after 15 seconds radiation whereas reduced to 92.8% after 60 seconds radiation. The effect of FeSO_4 is

minimal; it reduced the absorbance to 92.9% after 30 seconds radiation. Fig.2 shows that the effect of laser radiation at 1064 nm with a pulse rate of 3 Hz is minimal even in presence of element salts except CuSO_4 salt. Copper salt produced an increase absorbance to reach 125.9% after 30 seconds radiation.

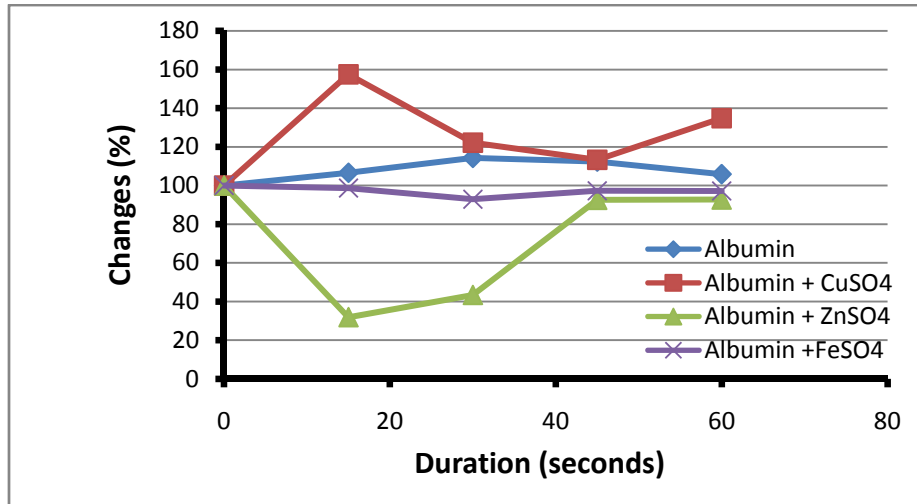


Fig. 1: Effect of Q switched Nd:YAG laser irradiation (532 nm) on the aqueous solution of human albumin (50µg/ml) in absence or presence elemental salts.

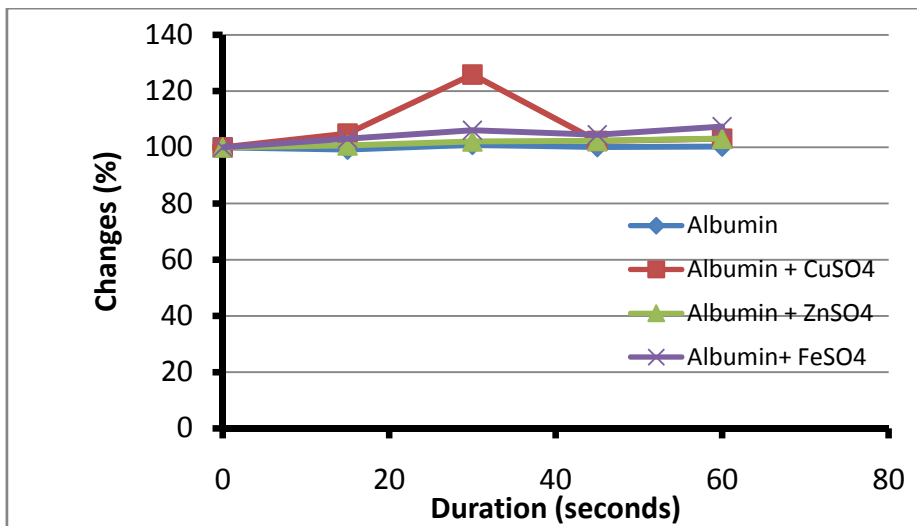


Fig. 2: Effect of Q switched Nd:YAG laser irradiation (1064 nm) on the aqueous solution of human albumin (50µg/ml) in absence or presence elemental salts.

2. Effect of laser radiation on the ctdsDNA aqueous solution

Fig. 3 shows the pronounced effect of laser radiation at 532 nm at 1 and 3 Hz pulse rate compared with 1064 nm

wavelength radiation. Using wavelength 532 laser radiation at pulse rate of 1Hz produced decrease absorbance of aqueous ctdsDNA solution (i.e. hypochromasia) to reach

84.8% after 40 seconds exposure to laser radiation. The opposite effect (i.e. hyperchromasia) observed when using 3 HZ in which the absorbance increased to 139.5% after 20 seconds radiation. Opposite effect observed

when wavelength radiation of 1064 used. At pulse rate of 1 Hz, there is no effect of laser radiation and at pulse rate of 3 Hz, the absorbance increased at exposure for 30 seconds (119.2%) and 40 seconds (110.8%).

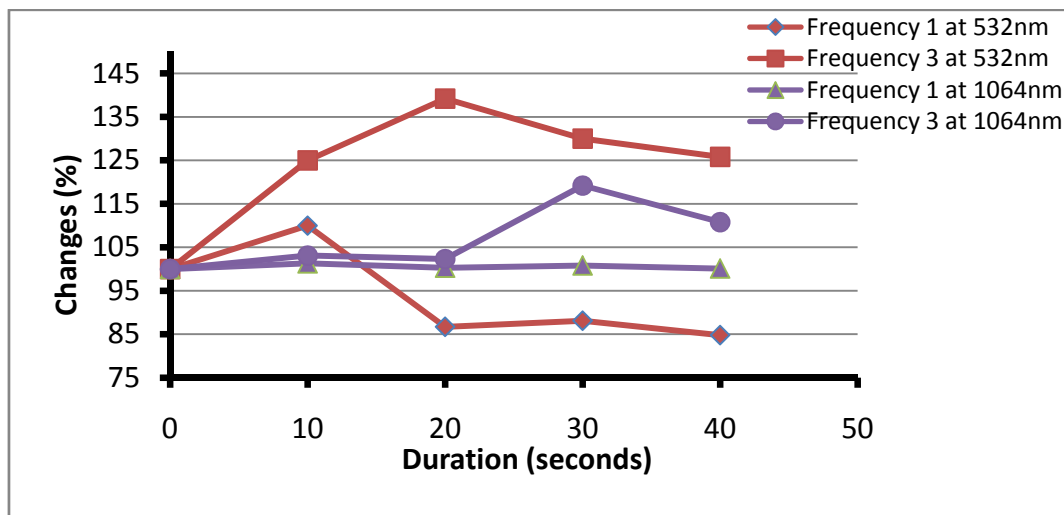


Fig.3: Effect of Q switched Nd:YAG laser irradiation (532 nm and 1064 nm) at different frequencies on the aqueous solution of calf thymus double strands DNA (50µg/ml) in absence or presence elemental salts.

Discussion

The results of this study show that Q-switched Nd:YAG laser irradiation produces specific alterations in the albumin and ctDNA molecules particularly at wavelength 532nm. An increase absorbance of aqueous albumin solution after 532 nm wavelength Q switched Nd:YAG laser irradiation, indicated destruction of albumin chain and render it to fragments of albumin peptides. This supports the notion that the effect of laser depends on the absorptive capacity of the exposed medium [10]. This finding is in agreement with other who found that laser irradiation of red blood cells produced changes in the shape of red cells due to changes in cellular protein content or configuration [11]. Fonseca and colleagues also demonstrated that exposure of blood to low-intensity laser alters the protein content in plasma at different fluencies

independent of the emission mode or frequency [12]. The effect of laser irradiation on aqueous albumin solution produced dual effects when incubated with copper-and zinc sulfate salts. Copper is a known oxidizing agent and it is more susceptible to breakdown by laser and this explained the higher value of absorbance of albumin solution with copper sulfate i.e. increase susceptibility to laser irradiation [13]. Laser irradiation of albumin solution incubated with zinc sulphate produced different effect in comparison with copper sulfate. In humans, serum zinc is primarily bound to albumin (80-85%). Its protective effect increases serum levels of total protein, albumin, which may be attributed to importance trace mineral zinc in metabolism of protein [14]. The current study also shows that the addition of ferrous sulfate salt stabilized the effect of laser on aqueous albumin i.e. the study add new

information about the role of iron salt in laser irradiation. The effect of laser irradiation at 1064 nm is inferior to that observed with 532 nm, depending on the type of lesion. This observation is in agreement with other study that showed concurrent application of the Q-switched 532nmNd:YAG laser followed the 1,064 nm laser is more effective removing the skin pigmentation than the Q-switched 1,064 nm Nd:YAG laser alone [15]. The variability in the effect of laser upon living molecules in respect to the wavelength and the pulse rate well observed in the irradiation ctdsDNA. The susceptibility of ctdsDNA is more to wavelength 532nm and higher pulse rate. Hypochromasic effect i.e. DNA strand breaks observed with wavelength 532nm radiation at frequency 3 pulse/nanosecond. Previous study observed the cleavage of dsDNA fragments in aqueous solution after irradiation with UV laser pulses at 193 nm i.e. both effects; hyperchromasia and hypochromasia demonstrated [16]. This concludes that using Q-switched Nd:YAG laser is safe upon the cellular elements at 1064 nm, pulse rate 1 Hz and spot size of 4 mm.

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