



Original Research Article

Effects of Laser Light on Vaccination with Hepatitis B Vaccine

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Abstract

Vaccination still the most important strategy in the prevention of infectious diseases, and the developing of powerful vaccine adjuvants is crucial to maximize the efficacy of these vaccines. Our objective was to introduce the laser adjuvant to increase the immunogenicity of Hepatitis B vaccine. Twelve wistar albino male rats were included in this study. The animals were divided into three groups; control, red laser, and infra- red laser groups. Rats were anesthetized by chloroform; the lower dorsal hair of the rats was shaved. The skin of laser groups was exposed next day to red laser ($\lambda = 635 \text{ nm}$) or to IR laser ($\lambda = 808 \text{ nm}$) for 60 seconds at 300mW after the rats were anesthetized similarly. The rats were then immunized by intradermal (i.d.) administration of (2 ug) of Hepatitis B virus surface antigen (HBsAg) into the laser-illuminated sites. Rats in control group were treated and immunized similarly except for no laser illumination. Laser therapies have diverse immunological effects; the most interesting effect is the change in the differential blood cells count after laser illumination which characterized by a significant increases in lymphocyte count and decreases in neutrophil count after one session of irradiation with red laser light. We found that 635 nm laser induce a significant increases in TNF- α level comparing with the other laser (808 nm) group or the control group. In conclusion the visible red laser has a significant boosting effect on HBsAg vaccine.

Key words : Hepatitis B vaccine, laser photobiostimulation, immunization, vaccination, laser adjuvant.

الخلاصة

لا يزال التطعيم هو الإستراتيجية الأهم لمنع الأمراض المعدية، إن تطوير مساعدات تطعيم فعالة يعد أمراً حيوياً لزيادة فعالية تلك اللقاحات. هدف عملنا إلى تقديم المساعد الليزري لزيادة فعالية التمنيع للقاح التهاب الكبد الفايروسي نوع ب. شملت الدراسة اثني عشر جرذاً ذكرًا نوع ويستربالينو. تم تقسيم الجرذان إلى ثلاث مجموعات: سيطرة، الليزر الأحمر، وليزر الأشعة تحت الحمراء. تم تخدير الجرذان بالايثر وتم حلق شعر المنطقة الظهرية السفلى. في اليوم التالي عرض جلد الجرذان إلى الليزر الأحمر (الطول الموجي 635 نانومتر) أو لليزر الأشعة تحت الحمراء (الطول الموجي 808 نانومتر) لمدة 60 ثانية بقدرة 300 ملي واط بعد تخدير الجرذ بنفس الطريقة السابقة. بعد ذلك تم تطعيم الجرذان داخل الجلد بـ (2 مايكروغرام) من المستضد السطحي لفايروس التهاب الكبد نوع ب في المناطق المشععة بالليزر. الجرذان في مجموعة السيطرة عوملت وطعمت بنفس الطريقة عدا أنها لم تشع بالليزر. كان للمعالجة بالليزر تأثيرات مناعية مختلفة، التأثير الأكثر إثارة للانتباه هو التغير في تعداد خلايا الدم التفرقي بعد التعرض لليزر والذي اتسم بالزيادة المعنوية في عدد الخلايا اللمفاوية ونقصان الخلايا المتعادلة بعد جلسة تعريض واحدة لليزر الأحمر. وجدنا كذلك أن الليزر ذو الطول الموجي 635 نانومتر سبب زيادة معنوية في مستوى عامل التئخر الورمي نوع الفا مقارنة مع مجموعة الليزر ذو الطول الموجي 808 نانومتر أو مجموعة السيطرة. كاستنتاج فإن الليزر المرئي الأحمر يمتلك قدرة تحفيزية ملحوظة على لقاح التهاب الكبد الفايروسي نوع ب.

Introduction

Vaccine development is one of the most cost-effective strategies for preventing viral infection that cause acute or chronic viral infection [1]. Vaccination is a biological preparation that stimulates immune response against some infectious diseases either bacterial or viral thus, enhances the active acquired immunity for specific disease, thereby, it is an effective way to prevent morbidity from infections and eradicate infectious disease [2, 3].

Historically, Vaccine adjuvants have been used to improve immune responses against non-living vaccines. The role of an adjuvant is to augment the effect of a vaccine via increasing antibody responses, inducing cell-mediated immunity, and thus providing intensified immunity to a specific disease [4, 5]. Several types of adjuvants have been developed with different modes of action. These include inorganic salts, oil emulsions, microbial derivatives, carbohydrates, liposomes, virus like particles and polymeric microparticle adjuvants and others [6]. The introduction of new adjuvants with potential effects on efficacy and safety is the new approach in the modern vaccines preparations [7].

The new generation of recombinant vaccines or synthetic antigens used nowadays is generally less immunogenic than previous attenuated or killed whole organism vaccines. This problem increased the need for more powerful improved sort of adjuvants for use with these vaccines [8].

Laser irradiations one of the recent strategies to be used with dermal vaccination to boost immune response via enhancing the function of antigen presenting cells [9]. Recent studies found that a short term laser irradiation of the vaccination site serves as a novel non-chemical vaccine adjuvant that can safely and efficiently enhance the humoral and cell mediated immune response via different routes mostly via

activation the motility of antigen presenting cells (APCs) as a result of the induction of the expression of specific chemokine in the skin which in turn increase the antigen (vaccine) uptake [9, 10, 11]. Laser biostimulation of immune response is believed to occur at very low irradiance and to belong to the group of photochemical interaction [12]. Laser irradiation at certain parameters stimulates the production of interleukins, tumor necrosis factors, interferons, and nitric oxide, and increases the activity of natural killer cells [13].

Hepatitis B virus is one of the most risky viral diseases that infect human worldwide [14]. Modern recombinant hepatitis B vaccines (HBsAg) are composed of purified molecules of hepatitis B "s" antigen (a glycoprotein of outer envelope). The adjuvant used is aluminium phosphate or aluminium hydroxide. The protective efficacy of this vaccine is related to the induction of anti-HBs antibodies, in addition to the activation of memory T-cells [15]. The aim of this study is to introduce the laser adjuvant to increase the immunogenicity of Hepatitis B vaccine.

Material and Methods

Animals

Twelve Wistar albino male rats, eight weeks of age with an average weight of 212 ± 15 grams were used in this study. All rats were housed in conventional cages in the animal facility of the college of medicine/Babylon University for at least two weeks before the experiment.

Laser devices

A CW red laser light ($\lambda = 635$ nm) with an adjusted output power of 300 mW and an IR laser light ($\lambda = 808$ nm) with an adjusted output power of 300 mW (Dragon laser, China) were used in this experiment. Average output powers were measured by a power meter (coherent, USA).

Immunizations

The rats were divided into three groups; control, red laser, and IR laser groups.

Rats were anesthetized by chloroform and the lower dorsal hair of the rats was removed by shaving. The skin of laser groups was exposed next day to red laser ($\lambda = 635$ nm) or to IR laser ($\lambda = 808$ nm) for 60 seconds at 300mW after the rats were anesthetized similarly. The rats were then immunized by intradermal (i.d.) administration of (2 ug) of Hepatitis B surface antigen (HBsAg) (LG life sciences, Korea) in a single dose into the laser-illuminated site. Rats in control group were treated and immunized similarly except for no laser illumination.

Blood Collection and Immunological Tests

Rats were sacrificed by overdose of chloroform. Blood sample were collected directly from the heart of each anesthetized rat before death. Blood films were prepared directly after blood collection and blood smear kept at room temperature for 24 hours before staining. Gimsa stain was used to stain the blood smear; Leukocytes were counted under microscope. Serum was prepared by centrifugation and kept at -20°C till analysis. Serum interferon- α (IF- α) and tumor necrosis factor- α (TNF- α) concentrations were evaluated using Rat IF- α and TNF- α ELISA Kits (Creative Diagnostics, USA) respectively according to the manufacture protocols.

Histological Examination

One centimeter of full thickness of the skin at the site of (laser illumination +vaccine) or vaccine injection was excised after 21 days of vaccination, fixed in 10% formalin and subjected to a standard histological examination.

Statistical Analysis

Data were analyzed using Sigmaplot version 12.0 software. Statistical significance was tested using a Student's t-test for unpaired observations. P values less than 0.05 were considered as statistically significant.

Results

Effects of Laser Lights on Leukocyte Production

Low power laser therapy has been shown to have diverse immunological effects. One of the most interesting effect is the change in the differential blood count and decreases in neutrophil values after few hours of irradiation with red laser light[16]. Our study aimed to investigate the long term effects of red and near infra-red (NIR) lasers on the differential blood count. Figure (1) show the effect of 635 nm red laser and 808 nm NIR laser on blood cells count after 21 days of i.d. vaccination with HBsAg only or skin pre illumination with 635 nm or 808 nm laser lights for 60 seconds at 0.3 W output powers.

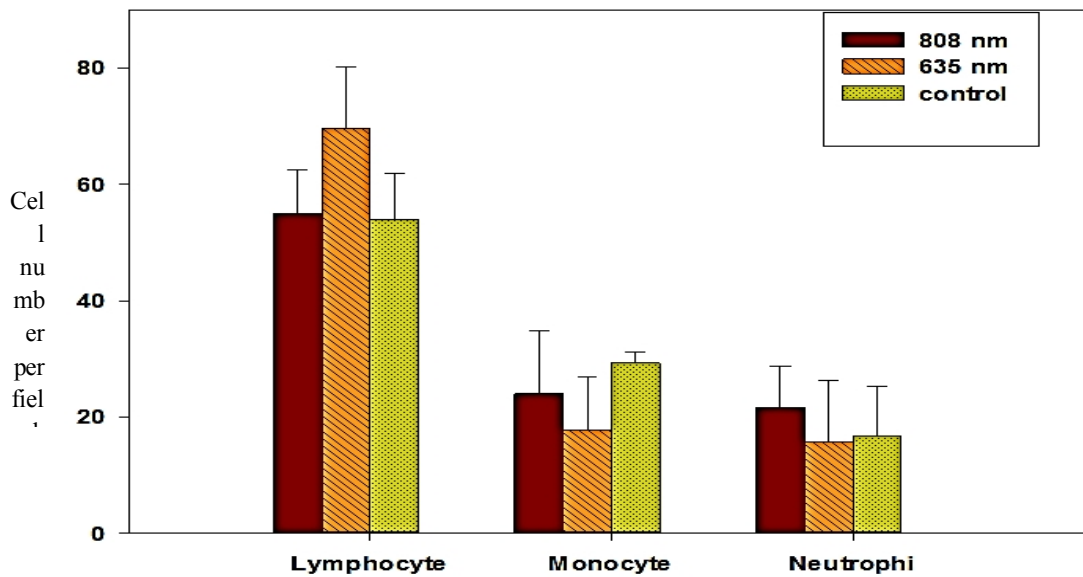


Figure 1: leukocyte infiltration in skin samples in control and laser treated skin samples shows a significant increase in lymphocyte migration at the vaccination site irradiated with 635 nm laser light at 45 J/cm² energy density

A significant increase was observed in lymphocyte number in the red laser treatment group (received the vaccine and the 635 nm laser) in comparison with control group (received the vaccine only) or NIR group (received the vaccine and the 808 nm laser) that show no significant change in lymphocyte count. Monocytes and Neutrophil showed no noticeable long term changes in all groups.

Effects of laser lights on leukocyte infiltration in skin

Antigen presenting cells are the primary targets for most vaccine adjuvants[17].

Thus, the first step in our work was to verified that laser treatment activate lymphocyte motility and aggregation in the site of administration. Figure (2) shows the lymphocytes aggregation in skin samples after 21 days of i.d. vaccination with HBsAgalone after skin pre illumination with 635 nm or 808 nm laser lights for 60 seconds at 0.3 W output powers. Results showed that laser treatment with 635 nm laser light significantly enhanced lymphocyte motility and confluence as appeared in the histologic images of rat skin samples.

Control

635 nm

808 nm

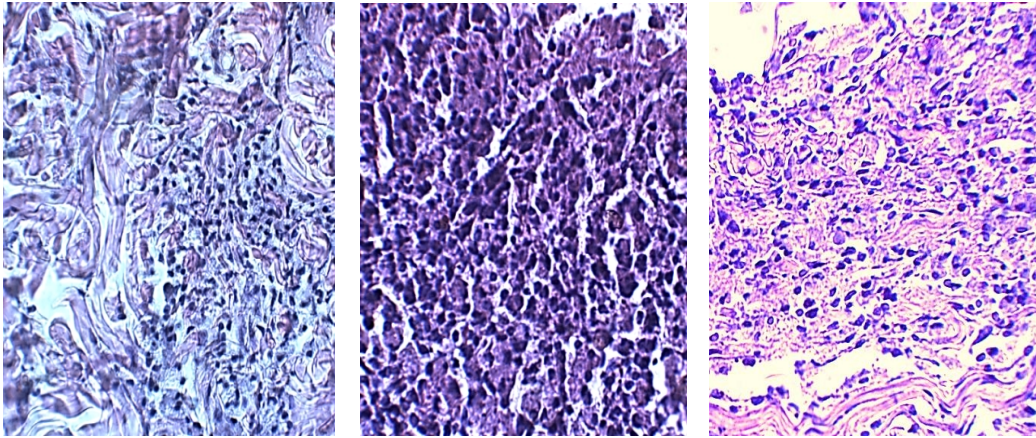


Figure 2: Full thickness of skin tissues stained with H&E stain (x40), show a significant increases in lymphocyte infiltration at the vaccination sites after the irradiation with 635 nm laser light in comparison with 808 nm irradiated site or the non-irradiated (control) site.

Effects of Laser Lights on Cell-Mediated Immune Responses

One of the essential mechanisms by which the vaccine initiates immune response is via the induction of cell-mediated immunity; this is the responsibility of the adjuvant [4]. In our study, laser light was used to trigger such response prior to the

i.d. injection with the vaccine. We found that 635 nm laser induce a significant increases in TNF- α level comparing with the other laser (808 nm) group or the control group as depicted in figure (3). In the other hand, laser pre-illumination didn't cause any marked changes in serum Interferon - α (IF- α) levels as shown in figure (4).

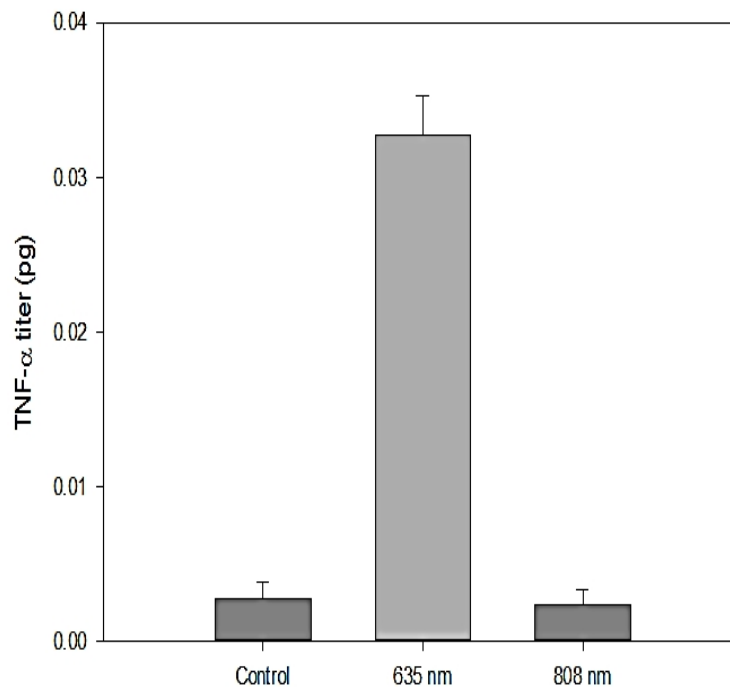


Figure 3: A significant increases in serum TNF- α after laser irradiation with 635 nm laser light at 0.3 W for 60 seconds in comparison with control and 808 nm laser irradiation at the same parameters.

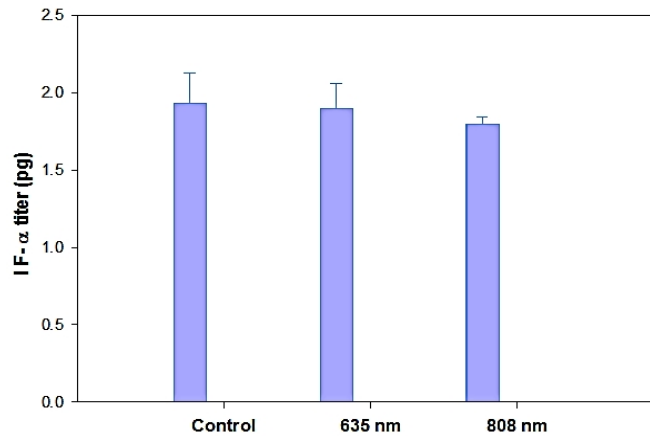


Figure 4: No significant increases in serum IF- α after laser irradiation with 635 nm and 808 nm laser light at 0.3 W for 60 seconds

Discussion

The main function of adjuvant in current vaccines is to maximize the immunological response and improve vaccine efficacy. Laser adjuvant was used in recent studies in the form of continuous wave near red laser as a safe, effective, low cost, simple to use with intradermal vaccine instead of conventional adjuvant[7]. The adjuvant action of laser is induced by brief illumination of a small area of the skin with non-invasive laser light prior to intradermal injection of vaccine[18].

Generally it is accepted that mitochondria play the major role in the cellular photobiomodulation particularly in the red and infrared regions. Components of respiratory chains like NADH-dehydrogenases and cytochrome c oxidase absorb laser light and get excited. This in turn will cause an alteration in the redox states and acceleration of electron transfer [19]. Cellular components of immune system are affected by laser light in the same way, causing lymphocyte proliferation [20], activation of macrophages in inflammation[21], marked increase in cytokines production [13], etc.

Laser adjuvant enhances the immunity system at the cellular level through increasing local inflammation, increasing the antigen presenting cells (APCs)

mobility[9], and stimulating proliferation of non-specific lymphocytes[22]. This may explain the increase in lymphocytes count after 635 nm laser irradiation even after 21 days of treatment.

Also, the release of tumor necrosis factor (TNF- α) in the 635 nm laser group was significantly ($p < 0.01$) greater than that in the other two groups as illustrated in figure (3). TNF- α is a pro inflammatory cytokine secreted by the peripheral blood mononuclear cells (PBMC)[20], its expression is strongly enhanced by laser irradiation[10]. Thus, 635 nm laser illumination of the skin caused this enhancement in TNF- α production. In our study, the irradiation with near infra-red laser showed no significant change in TNF- α level, although, in previous study with 780 nm laser light it was found that TNF- α tend to decrease after few days of irradiation. Because TNF- α is a pro-inflammatory cytokine, it is possible that it released at a higher concentrations in the initial stages of the treatment [21]. Near infra-red light penetrates deeply into the skin due to its low absorption by the living tissue including immune cells[22]. Thus, the effect of red laser at certain dose will be higher than that of infra-red laser at the same dose since the photobiomodulation depends mainly on the amount of energy absorbed by the cellular components.

The energy density or laser dose used in this study is equivalent to 45 J/cm² which is much higher than most energies used in previous studies. It was found that the elevation in the production of certain interleukins induced by low level laser irradiation is disappeared when the laser dose exceed certain threshold close to that used in our study[16]. Previous studies on the effect of low level He-Ne laser on the gene expression of IF- γ detected a significant inhibition in this gene after laser irradiation at higher doses[23]. This may explain the hypo-secretion of IF- α by PBMCs after irradiation with both types of lasers.

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