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Photoimmunotherapy by Cortactin monoclonal antibody conjugated with Hematoporphyrin derivative of a subcutaneous murine mammary adenocarcinoma using low power He-Ne laser

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Abstract:

Photoimmunotherapy (PIT) is a promising approach which aims to increase the specificity of photosensitisers used in photodynamic therapy through its conjugation to monoclonal antibodies (MAb) directed against tumour antigens. The current study aims to increase and improve the therapeutic selectivity of photodynamic therapy (PDT) by conjugating the HpD to specific (mAb) in order to target one of the specific antigens of mammary adenocarcinoma. a screening has been done to detect specific antigens on mammary adenocarcinoma cells and we found that Cortactin is the unique antigen on tumor tissue in our model, then anti-cortactin mAb was linked to HPD, used for not only improve specificity, but vastly enhances tumor cell killing when they are photo-immunotargeted by HpD-monoclonal antibody conjugates comparing with unconjugated HPD and then exposed to low power He-Ne laser (632 nm). Female mice, which were transplanted with AM3 (mouse mammary adenocarcinoma transplantable tumor line), were randomly divided into five groups of 10 mice each; the first group was treated by PIT using HpD conjugated with Cortactin, in comparison with HpD alone in a second group. The third and fourth were control groups with and without HpD alone respectively. Tumor growth indices and metastasis incidence were calculated. Photoimmunotargeting by HpD-mAb conjugates activated by a low-power He-Ne laser showed significant tumor growth inhibition similar to PDT using unconjugated HpD activated by He-Ne laser, whilst control groups were without any significant effect. The resultssuggest that conjugation was with low quantity of the HPD or not stable enough and this need further studies.
keyword: *Cortactin monoclonal, mammary adenocarcinoma.*

Introduction:

Photodynamic Therapy (PDT) is a multi-factorial anticancer therapy that kills cancer cells by the photochemical generation of reactive oxygen species following absorption of appropriate wave length of laser by a photosensitizer, which selectively accumulates in tumor cells [1,2,3,4,5]. In Oncology field, PDT is basically used for the treatment of superficial tumours, where the irradiated area is accessible, and therapy is associated with high safety index and favorable tumour response [2, 3]. PDT has also been investigated for the treatment of deep-seated or disseminated malignancies [4]. Unfortunately, acute damage to surrounding normal tissue and treatment-related toxicity is often observed as a consequence of poor photosensitiser selectivity, and difficulties directing the light source to the tumour mass [5]. Many techniques have been tested to raise the specificity of photosensitising agents for cancerous cells [6]. Efforts to activate the photodynamic effect have led to the use of certain biological molecules as carriers of photosensitizers to enhance the photosensitizer selectivity [7]. One very attractive way to gain this is by conjugation of the photosensitizing agents to monoclonal antibodies (mAb) which are specific for tumour-associated antigens [8]. Several studies have illustrated the possibility of conjugating photosensitizers to a variety of antibodies for tumour targeting. This technique is often termed photoimmunotherapy (PIT), in which mAb and photosensitiser are attached to the carrier molecule to increase both photosensitiser loading and conjugate solubility. The photosensitiser is used without purification and contains three carboxyl groups which could lead to crosslinking of the antibody or polymer. The use of a polymeric carrier may also affect the photophysics of the photosensitiser and can lead to nonspecific uptake [9].

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One of the most important substances for PDT is hematoporphyrin derivative (HPD) which has been shown to selectively localize in malignant tissues. Generally, porphyrins are powerful photosensitizing agents that can cause destruction and death of malignant tissues in which they have localized by the generation of singlet oxygen when activated by the red light emitted from Helium–Neon laser (He–Ne laser, λ = 632.3 nm) [10, 11]. In a previous studies we have shown that the HpD-mediated PDT leads to inhibition of AMN3 (mouse mammary adenocarcinoma tumor cell line) using He–Ne laser in vitro [12] and in vivo [13].

In this study, the conjugation of hematoporphyrin to cortactin as a mAb has been investigated. HpD- Cortactin conjugates have shown a destruction action of cancerous cells when they photo-activated by irradiation emitted by low-power He-Ne laser. The conjugation of a monoclonal antibodies such as cortactin with HpD that preferentially binds to tumor cells offers promise as a therapeutic agent that will not only improve specificity, but vastly enhances tumor cell killing when exposed to light in its photodynamic range. The application of the monoclonal antibody-hematoporphyrin conjugate squamous cell carcinoma cells in vitro, followed by exposure to light in about 632 nm range, increases the kill ratio by a factor of 10 times (14). Thus, we applied this technology (PIT) to increase selectivity and efficiency to PDT using He-Ne laser.

MATERIALS AND METHODS:

Experimental animals: Swiss white female mice, 12 weeks old, weighing 20-25 g were used. They were provided with food and water. A transplantable mammary adenocarcinoma cell line named AM3 (provided by Dr.Ahmed M. Al-Shammari/ experimental therapy department/Iraqi Center for Cancer and Medical Genetic Research) (15) was propagated by serial transplantation into female Swiss white mice. Tumor material for inoculation was obtained by sterile aspiration to the flank tumors. A 0.25-mm3 sample of macroscopically viable tumor, which is equal to approximately 2×106 cells, was injected S.C. under the dorsal flank of each mouse. The take rate of the tumors following transplantation was nearly 80-100%. Under these controlled conditions the implant size did not vary by more than 10%.

Preparation and administration of HPD: The photosensitizer, Hematoporphyrin derivative-HCl (HPD) was purchased from Sigma-Aldrich Chemical Co. (Germany). The hydrochloric salt of HPD was completely dissolved in phosphate buffer saline (PBS; pH 7.2) in dark chamber at a concentration of 100 mg/ ml [17], after completely dissolved by vigorously shakening with a vortex mixer for 5 min at 37 ○C.

Preparation of HpD -mAb conjugates: Hematoporphyrin derivative (HPD) was conjugated with a murine monoclonal antibody (Cortactin Abcam, UK) against murine mammary adenocarcinoma through bovine serum albumin (BSA) (US-biological, USA) as an intermediate. The molar ratios of the conjugates mAb Cortactin-BSA-HPD were 1:200, respectively [16].

Laser and irradiations: The source of laser used in this study was Helium-Neon atomic gas laser (Model DL30, LG Lasers). The wavelength of light that emitted from this laser and output power were determined using a handheld laser power meter (Edmund Optics Inc., Portland, USA).The wavelength was 632.8 nm (red light) and output power equal to 20mW (mill watt) as a continuous wave. The dose of laser was 31.5 J/cm2. The light was focused into a 7mm diameter light spot, producing a of uniform irradiation treatment area.

Treatment protocol for HpD-mAb and HpD-based PDT: Ten days after AM3 tumor implantation, when tumors reached the appropriate size of 0.5-0.7 cm3, they were randomly divided into five groups of 6 mice each.

The first group intratumorally injected with mAb-HpD and the second group intratumorally injected with HPD 30 mg/ kg of body weight and both of them superficially irradiated with low power He-Ne laser just 24 h after HPD administration, The total laser dose at the irradiation area which encompassed the tumor and 1–1.5 mm of the surrounding skin was 31.5 J/cm2. The third group intratumorally injected with HPD only without irradiation. The fourth group received irradiation without HPD administration and the last group left as control without administration and irradiation.

Assessment of tumor response: In all experiments, the tumor growth was recorded every 2 days by measuring a perpendicular diameters using vernier caliper. Tumor volumes were estimated using the following formula according to (17):

Tumor volume (mm3) = a.b2/2

a= length of tumor mass (mm), b= width of tumor mass (mm)

Relative tumor volumes (R.T.V.) and Tumor growth inhibition (GI%) were calculated using the following formulas according to (18):

$$R.T.V(\text{day } x) = \frac{\text{tumor volume}(\text{day } x)}{\text{tumor volume}(\text{day } 0)} \times 100$$

$$GI\% = \frac{\text{tumor volume of untreated group} - \text{tumor volume of treated group}}{\text{tumor volume of untreated group}} \times 100$$

To assess the response to treatment an index was defined: DX (tumor volume X days after PDT/tumor volume before PDT).

Statistical analysis: The unpaired t-test was used to establish the significance of differences between groups. Differences were considered statistically significant when $P \leq 0.05$.

RESULTS:

The present study investigated the effect of laser irradiation on the growth of a subcutaneous implanted AM3 mammary adenocarcinoma tumor after intratumoral application of HpD and or anticortactin mAb linked to HPD. Tumor response was evaluated following tumor growth and growth inhibition studies.

Effectiveness of HpD-based PIT in delaying tumor growth: The effectiveness of HpD as a photosensitizer for PIT was determined by assessing the extent of tumor growth after one PIT application. Two response indices were defined, measuring the ratios between tumor volumes before and after treatment. After a single application all indexes were lower than those of the untreated tumors, indicating that if not a complete reduction, a delay of tumor growth occurs. Irradiation on HpD injected intratumorally alon or with cortactin mAb appeared to induce a greater reduction in tumor volume earlier. An index of 57 was observed at 4D and 70 at 8D, but differences between times were not statistically significant. In order to compare the response of PIT between groups, tumor growth curves were used (Figure-1). Animals received one intratumoral mAb- HpD and or HpD - PIT alone; a single application induced a clear tumor growth delay for intratumoral HpD administration which continued to the end of the experiment at day 20.

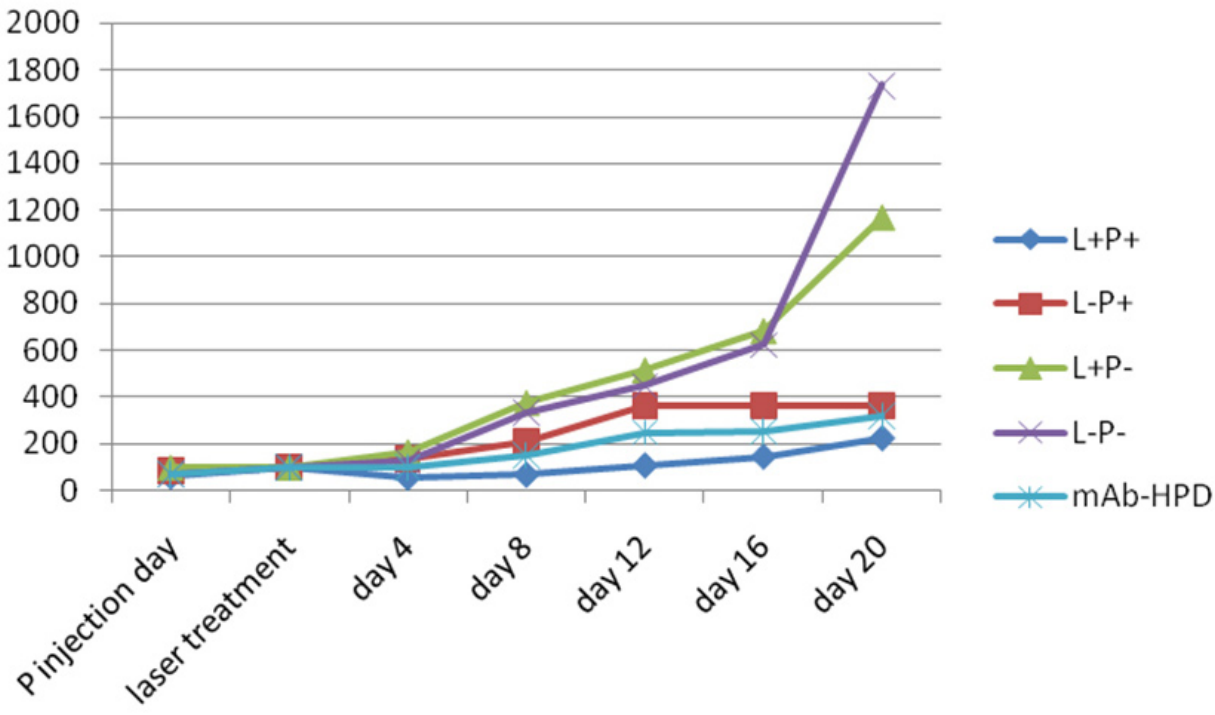

Figure-1, Tumor growth curve of treatment groups, group one of photosesitizer linked to anticortactin mAb, the second group of photodynamic therapy which cause marked growth inhibition in compare to photosensitizer alone or laser alone which show no response or delay in tumor growth, especially laser group which have similar growth pattern to control untreated group.
mAb-HPD = anticortactin mAb conjugated with HPD
L+P+ = Laser and photosensitizer treatment at the same time
L-P+ = Photosensitizer treatment alone
L+P- = Laser treatment alone
L-P- = control without treatment

DISCUSSION:

According to a previous study, photodamage effects were observed after photodynamic action in vitro, when AMN3 cells were incubated with different concentrations of HpD and then illuminated with different doses of He-Ne laser in vitro [12]. The current study showed the using intratumoral PIT in vivo for treatment a subcutaneous murine mammary adenocarcinoma by the combination of anti-cortactin mAb-HpD and unconjugated HpD with low power He-Ne laser, can induce killing of tumor cells through photo damaging exactly with the same effect of regular HPD alone which can induce more effect by increasing the conjugation effeciancy. Photodynamic actions including photoimmunotargeting is the efficient selective technique for killing of target cells which exposed to certain photosensitizers that is linked to a specific unique antigen on tumor cell surface and corresponding laser light with appropriate wavelenght [19, 20]. The laser-

activated process necessarily requires the presence of a light-absorbing substance, the photosensitizer HpD, which initiate photochemical processes in a non-absorbing substrate (AM3 cells). The pathway which involved a photosensitizer triplet state reacted initially with a substrate rather than molecular oxygen, this is termed Type I photochemical reaction. In the alternative Type II photochemical reaction the photosensitizer triplet state reacts first with molecular oxygen producing a singlet oxygen (1O2), it is the main free radical ion which responsible for cell death [6,7]. The most important photosensitizer is HpD which localizes and retains in tumors anywhere in the body after intravenous administration because it is conjugated to a specific monoclonal antibody for tumor antigen. There is no therapeutic effect until HpD in tumor tissue is exposed to appropriate visible light, which is usually the red region of the electromagnetic spectrum. The irradiation exposure induces necrosis followed by sloughing of the necrotic tissue and re-growth of normal tissues. The putative action mechanism in PIT is 1O2 generated by energy transferring from the HpD triplet state to tumor oxygen, and then initiated a lipid peroxidation in the endothelial cells of the small blood vessels which supply the tumor cells with blood. The tumor oxygen supplying is blocked by this process afterward the observed necrosis is induced. Direct cancer cell killing is involved as well by inducing cell injury (necrosis) and apoptosis. Interestingly, oxygen is required for the initial photochemical reaction and its depletion initiates the clinical response. The selectivity mechanism is depended on the total amount of HpD in tumor tissue which were several times higher than normal tissue, that is due to thier ability to accumulate in malignant tissue [21 & 22], which is intradermally located murine adenocarcinoma.

In summary, our finding in the current study showed the possibility of using PIT in conjugation with mAbs to target tumor cells but it need for more efficient method for conjugating the HPD to the mABs and this can be considered as new antitumor modulation for breast cancer model in mice bearing murine mammary adenocarcinoma where there were interesting antitumor activity which can lead to clinical applications.

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العلاج المناعي الحركي الضوئي للخلايا السرطانية باستعمال الاجسام المضادة الاحادية النسيلة المرتبطة بالمحسس الضوئي

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الخلاصه:

يعد العلاج المناعي الضوئي طريقة واعدة تهدف لزيادة انتقائية المحسسات الضوئية المستعملة في العلاج الحركي الضوئي من خلال الاقتران مع جسم مضاد احادي النسيله والذي يوجه ضد مستضد ورمي. هدفت الدراسة الحالية الى زيادة وتحسين الانتقائية العلاجية للعلاج الحركي الضوئي من خلال اقتران مشتقات الهيماتوبيرفورين مع جسم مضاد احادي النسيله يستهدف مستضد معين خاص بسرطانة الغدة اللبنية. تم اجراء مسح للمستضدات الخاصة بسرطانة الغدة اللبنية ووجد ان الكورتاكتين هو مستضد فريد على النسيج الورمي في نموذجنا المدروس. تم اقتران الجسم المضاد مع مشتقات الهيماتوبيرفورين لتحسين الانتقائية وبالتالي قتل الخلايا السرطانية عندما يتم استهدافها بالجسم المضاد المقترن مع المحسس الضوئي وتعريضها بعد ذلك لليزر ذو القدرة الواطئة. تم غرس فئران اناث بورم الغدة اللبنية الفاري ووزعت عشوائيا على خمس مجاميع من عشرة فئران, تم معالجة الاولى بالمحسس المقترن والليزر في حين لم تعرض المجموعه الثانية لليزر وجرعت المحسس المقترن فقط. المجموعه الثالثة اعطيت فقط محسس والرابعه تركت كمجموعة سيطرة. تم قياس نمو الورم ووجد ان للمحسس المقترن تاثير قوي مشابه لغير المقترن وان لم يكن اكثر تاثيرا وذلك ربما يعزو لقلة المحسس المرتبط او لعدم استقرار الارتباط وهذا يحتاج الى دراسات اكثر.