

Treatment of cancer by low intensity laser radiation therapy

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Abstract: Cancer treatment is one of the major problems facing the world year after year. The most common used for cancer treatment is chemotherapy, as it is a narcotic toxin that is applied to the patient, which destroys the cells of the body and thus body organs. It not only kills cancer cells but also normal cells, which cause side effects such as blood toxicity and decrease in the number of red blood cells, white blood cells, platelets and thus anemia, feeling of vomiting, diarrhea, loss of hair and nails. Photodynamic therapy (PDT) is one of the relatively new ways to treat malignant tumors with minimal patient side effects. It depends on photon activation by exposure to light, the light used in this research is laser therapy to supply adequate light dose of the energy for a limited time to stimulate the photosensitizer, but not enough to damage neighboring healthy tissue at a specific wavelength in the presence of O₂. In this work photodynamic therapy modality was used, Chlorin E6 was used as a photo sensitizer drug and one source of energy were used; namely low intensity laser therapy with three frequencies levels (1000, 2000, and 3000 Hz). Tumor-bearing animals were divided into the following groups each of 10 animals. The 1st group consists of two control groups: the 1st one was untreated group and the 2nd one was injected with photosensitizer alone. The 2nd group consists of 30 mice divided into 3 sub-groups and tumor site was irradiated with laser light as follows: 1000 Hz, 2000 Hz and 3000 Hz for 5 min. 3rd group consists of 30 mice injected with Chlorin E6 15 mg/Kg, then divided into 3 subgroups and tumor site was irradiated with laser light at the same conditions of 2nd group. The results show decrease in tumor size treated with drug which is used as a photo sensitizer, laser, and combined treatment, both in the presence and absence of photosensitizer. The most effective treatment is to use drug and laser together than used the medicine or laser alone. Also the presence of photosensitizer increases the effect of laser on tumor size. However, from histopathology of Ehrlich tumor using 3000Hz of laser irradiation in the presence of the photosensitizer drug approximately has the most effect than using the photosensitizer drug alone and exhibit totally necrotized tumor. It can be concluded that the use of photosensitizer alone has no effect on Ehrlich's tumor. However, the presence of the drug with exposure to 3000 Hz infrared lasers has had an effective effect on the tumor. This work requires more effort to use phototherapy with exposure to different energies of infrared lasers.

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1. Introduction

Cancer remains leading cause of death globally which is a multigenic and multicellular disease that can arise from all cell types and organs with a multifactorial etiology. Cancer treatment is one of the main challenges that face the scientific centers all over the world. The cancer incidence percentage increases year after year. The most used treatment for cancer is chemotherapy which is an application of systemic cytotoxic drug inside the patient. There are two main problems in chemotherapy that the cytotoxic drug is given systematically and cannot be localized in the tumor region in most cases that lead to killing the cancer cells as well as normal cells; and that cause the chemotherapy side effects like anemia, decrease in all blood cells count and platelets, nausea, vomiting, diarrhea, hair and nails loss and may cause a kind of blood toxicity.

The goal of cancer treatment is to produce cure by removing the cause of the disease while producing

as little harm to the patient as possible. Most conventional cancer treatments, e.g., surgery, radiation and chemo-hormonal therapy are possible, but they are usually highly morbid with low success rates⁽¹⁾. The dilemma of cancer therapy lies in selectively targeting and destroying only the cancer cells and leaving the normal cells unharmed. For this reason, new areas of cancer research have explored more specific methods of targeting only cancer cells. Such methods include development of new classes of drugs that are directed to unique sites on cancer cells, as well as techniques that specifically tag only cancer cells for destruction. The destruction of solid tumors using photodynamic therapy and laser therapy has been under investigation for this purpose⁽²⁻³⁾.

Photodynamic therapy (PDT) is a relatively new approach for the treatment of malignancies with only minimal side effects for the patient. It is based on the administration of a tumor-localizing dye photosensitizer (PS) that becomes toxic to neoplastic

cells when is activated by light usually from a laser at a specific wavelength in the presence of O₂. PDT is a two-step procedure: first a photosensitizer is administered, and then the region where the photosensitizer accumulates is exposed to light of a specific wavelength, which activates the photosensitizer⁽⁴⁻⁵⁾, PDT is based on the fact that photosensitizers are absorbed by all cells, but are selectively retained by malignant tissue⁽⁶⁾. PDT kills tumor cells *via* apoptosis or necrosis (or both), both *in vivo* and *in vitro*. The particular mode of cell death in response to PDT depends on experimental conditions, such as the dose of PDT and the subcellular localization of the photosensitizer⁽⁷⁾. Furthermore, PDT-mediated apoptosis may have different mechanisms depending on the type of cells being treated, the type of photosensitizer being used, the light delivery protocols employed, and the time lag between the photosensitizer and light treatment^(8,9).

PDT is a promising modality for the treatment of solid tumor and other non-malignant conditions. PDT is a minimally invasive technique that avoids many of the side effects typical for radiation and chemotherapy, since the drug and light by themselves are not cytotoxic. It has the ability to irradiate only tumor, the possibility of treating multiple lesions simultaneously and the ability to retreat a tumor in order to improve the response⁽¹⁰⁻¹¹⁾. The treatment involves administration of a photosensitive drug to the target site and after a suitable time period, to allow the drug to accumulate within the tumor, the drug is activated by specific energies of visible light. Laser, near-infrared region, is typically used to produce the activating light, which has greater penetration through tissues and can be selected to lie beyond the longest single-photon absorption band⁽¹²⁾. When the photosensitizer is illuminated with light of appropriate wavelength, the molecule becomes excited. Photo excitation of photosensitizer leads to the formation of singlet oxygen (¹O₂) and it was suggested that, this highly active oxidant is the main damaging agent in PDT⁽¹³⁾. However, there are some indications that besides O₂ other reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂), superoxide (O₂⁻) and hydroxyl (OH) radicals might be involved in the PS-PDT induced tumor eradication⁽¹⁴⁻¹⁵⁾. Investigations on the mechanism of action of PS-PDT showed that this treatment modality may include not only a direct damaging of organelles within malignant cell, but also destruction of blood vessels in the tumor locus resulting in reduced oxygen supply, nutrients^(16,17) and possibly activated the immune system⁽¹⁸⁾. Some drugs, referred to as photosensitizers, have synergistic effect with red light irradiation. The majority of photosensitizers are Chlorin E6, the most studied and best known photosensitizer used in PDT in many

countries worldwide, possesses clear structure, better tumor targeting ability, strong photodynamic activity and lower cytotoxicity^(19,20,21), which have been well investigated in PDT. Chlorin E6 is known to enhance the cell-killing effect of light irradiation at a dose at which the chemical alone has unknown biological effect. The present work aims at studying the effect of a combined treatment of low intensity laser radiation with and without photosensitizer e.g. Chlorin E6 on tumor growth rate. In this work photodynamic therapy modality was used. Chlorin E6 was used as a photosensitizer drug and one source of energy were used; namely low intensity laser therapy with three frequencies levels (1000, 2000, and 3000 Hz) in order to study their effects on Ehrlich tumor without any side effect on normal cells.

2. Material and Methods

2.1. Specifications of laser unit

Fig.1. show photo of low intensity laser therapy unit (Mustang, 2000, Germany). The maximum power is 2mWatt, the emission frequencies ranged from 10Hz to 3000Hz and two out puts, two laser emitters can be connected simultaneously.



Fig.1. Photo of low intensity laser therapy unit

2.2. Experimental procedures of PDT

2.2.1. Source of Photosensitizer

photosensitizer Chlorin E6 was purchased from Sigma Chemicals Company. A commonly used photosensitizer CAS Number: 19660-77-6, Purity: ≥95% Molecular Weight: 596.68. Molecular Formula: C₃₄H₃₆N₄O₆.

2.2.2. Ethics and cancer cells .

Ehrlich ascites tumor was chosen as a rapidly growing experimental tumor model⁽²²⁾ where various experimental designs for anticancer agents can be applied. Ehrlich ascites carcinomas cells (1×10⁶cells),

obtained from the animal house of National Cancer Institute NCI Cairo University. Ascites fluid was collected on the 7th day after injection. The Ehrlich cells were washed twice and then resuspended with 22–25g body weight and 6–8 weeks old and were then injected subcutaneously in their left flanks where the tumors were developed in a single and solid form. Tumor growth was monitored post-inoculation until the desired volume was reached. All animal procedures and care were performed using guidelines for the Care and use of laboratory animals⁽²³⁾ and approved by the Animal Ethics Committee at Cairo University⁽²⁴⁾.

2.2.3. Experimental animals

A total of 80 male Swiss albino mice with age 60–65day, weighing 20 ± 2.5 gm, were used. Ehrlich ascites carcinoma cells 2×10^6 mammary in origin, diluted approximately in 0.9% saline were inoculated subcutaneously on their left flanks region of mice. The animals were housed in plastic cages and were kept under natural light with diet and water at available. When the tumor had grown to about 10 mm in diameter at day 7 after inoculation, the treatment study was started.

2.2.4. Preparation of photosensitizer Chlorin E6

Photosensitizer Chlorin E6 was dissolved in a sterilized saline solution 0.9% saline and administered to tumor-bearing mice at a dose of 15mg/kg by injection for 15 day 24 hours before exposure to different treatment modalities.

2.3. Methods of treatment

Tumor-bearing animals were divided into the following groups each of 10 animals.

1- 1st group two control groups (20 animals): the 1st was untreated group and the 2nd was injected with photosensitizer alone.

2- 2nd group consists of 30 mice divided into 3 sub-groups and tumor site was irradiated with laser light as follows:

a- 1000 Hz, for 5 min.

b- 2000 Hz for 5 min.

c- 3000 Hz for 5 min.

3- 3rd group consists of 30 mice injected with photosensitizer Chlorin E6 15mg/Kg, then divided into 3 subgroups and tumor site was irradiated with laser light at the same conditions of 2nd group

2.3.1. Laser Treatment

The mice were anesthetized by Ether and the hair over the tumors was removed. The mice were fixed on a plate of cork with the tumor lifted up and then the laser probe was placed tightly to irradiated tumor with laser for a period of five minutes.

2.4. Tumor size measurements

Due to the high growth rate in Ehrlich tumor model, change in tumor volume (Vmm^3) was monitored over a 14day period for laser-

photosensitizer treated groups and control group. Ellipsoidal tumor volume was assessed every day and tumor volume (V) was calculated using the formula

$$V = (\pi/6) (d)2 (D) \quad (1)$$

Where D and d are the long and short axes respectively. Tumor diameters were measured with a digital Vernier calipers. Mice were selected for treatment when the tumors reached the desired volume ($0.7-1cm^3$). This size was chosen as a convenient treatment size that matches the laser spot size. Multivariate analysis of variance (MANOVA) was carried out to investigate the effect of both time (from day 0 up to day 15) and type of treatment on tumor growth. T-TEST (least significance difference) multiple-comparison test was also conducted to check the significance between group pairs⁽²⁵⁾.

2.5. Inhibition ratio percent of the tumor size

After two weeks of treatment with photosensitizer, mice were slaughtered, tumor was removed and weighted with grams then calculated the reduction in the percentage of tumor by the following equation⁽²⁶⁾.

$$Tumor\ Volume = \left[\frac{C - T}{C} \right] \times 100\% \quad (2)$$

Where C & T is the average tumor weight of the control and treated group respectively.

2.6. Tumor extraction

The tumor mass was collected and its measure at its longest and shortest axis was determined using a digital caliper. Then the solution was maintained at 10% formaldehyde for one day and transferred to 70% alcohol. After that the samples were embedded in paraffin, cut at $5\mu m$ thick and stained with hematoxylin-eosin (HE) for light microscopic observation⁽²⁷⁾.

2.7. Histopathological examination

At the time of sacrifice, the tumors were excised from the animals samples from each tumor tissues were fixed in 10% formalin and embedded in paraffin. Sections of tissues were stained with Hematoxylin and eosin⁽²⁸⁾.

2.8. Statistical Analysis of Data:

The data were analyzed using one-way analysis of variance (ANOVA). Results were expressed as mean \pm standard error (SE) and values of $P > 0.05$ were considered non-significantly different; while those of $P < 0.05$ and $P < 0.01$ were considered significant and highly significant respectively probability expresses the general effect between groups.

3. Results

Fig2. & Fig3. Show the variation of tumor volume as a function of treatment periods. The results showed a difference in the size of the tumor during different periods of treatment which showed a clear decrement in the size of the tumor when use the drug

alone or laser beam alone or both together also results showed that the treatment of ehlich tumor with laser beam at the frequency of 3000 Hz and drug are more effective in the destruction of cancer cells than using of laser or drug separately.

As shown in Fig.4. After the completion of the treatment period, the size of the tumor was measured for different groups by increasing the intensity of the laser beam. The results showed a decrease in the size of the tumor and this decrease was decreased in the presence of the medicine with radiation.

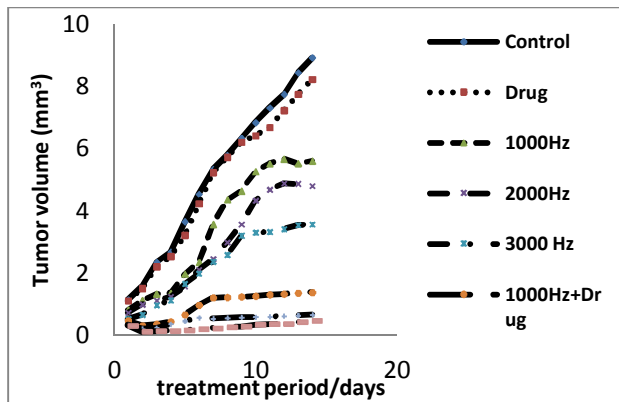


Fig.2. Tumor volume as a function of treatment periods for the different modes of treatments.

Tumor growth rate after 14 days of starting treatment of different groups are presented in table (I). It is clear from this table that there was not significant changes in tumor size by using the drug alone which has no any effect on the tumor size along the total period of treatment. However, there is very high significant effect by using 3000Hz of laser radiation in the presence of Chlorin E6 drug which is the most effect than using the drug alone.

Table (I): Tumor growth rate after 14 days of starting treatment of different groups

mice no	Tumor growth rate (mm ³ /day)		
	Control group	Chlorin E6 drug only	Laser (3000Hz)+Chlorin E6 drug
1 st	57.8	50.9	0.025
2 nd	63.3	55.2	0.027
3 rd	68.4	61.2	0.033
4 th	73.1	68.5	0.035
5 th	77.5	72.3	0.036
6 th	84.4	74.5	0.056
7 th	89.3	76.6	0.063
Mean value	73.4	65.6	0.0393
±SE	4.257	3.771*	0.0055**

* Not significant **very high significant

Table II illustrates Tumor inhibition ratio % at the end of 14 days starting from the day of treatment. It is clear from this table II that the inhibition ratio

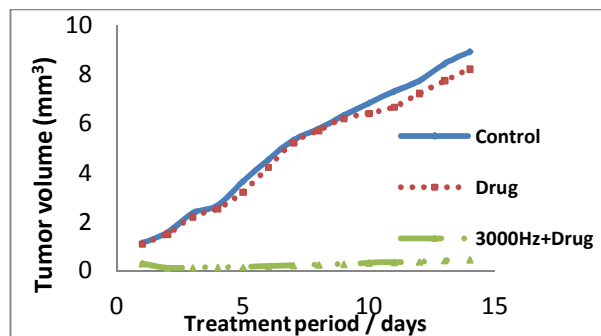


Fig.3. Tumor volume and the period after starting the treatment with combination of laser and drug.

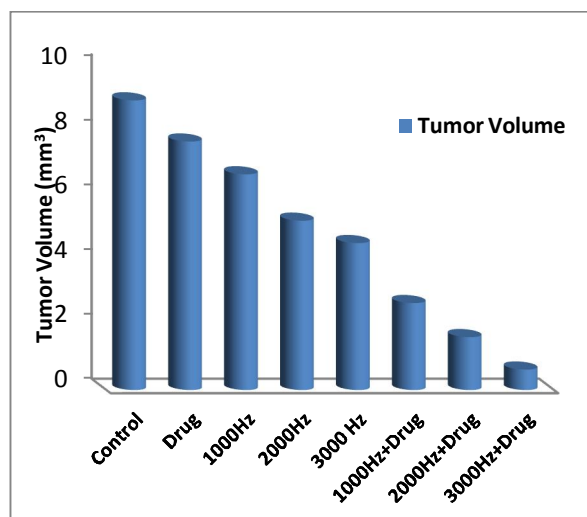


Fig.4. Tumor growth at day 14 of treatment as a function of frequency (Hz) of laser.

percent increases from 2.9% for drug only to 81.75% for using laser (3000Hz) and Photosensitizer drug together which is the most effective effect.

Table (II): Tumor inhibition ratio % at the end of 14 days starting from the day of treatment

Groups	Tumor weight (gm)	Tumor inhibition ratio (%)
Control	1.37 ±0.034	0
Chlorin E6 (drug)	1.33 ±0.054	2.9
Laser frequency (Hz)		
1000	1.27±0.053	7.3
2000	1.24 ±0.011	9.49
3000	1.18 ±0.013	13.87
Laser frequency (Hz)+ Chlorin E6 Drug		
1000	1.14 ±0.044	16.79
2000	0.75 ±0.011	45.26
3000	0.25±0.068	81.75

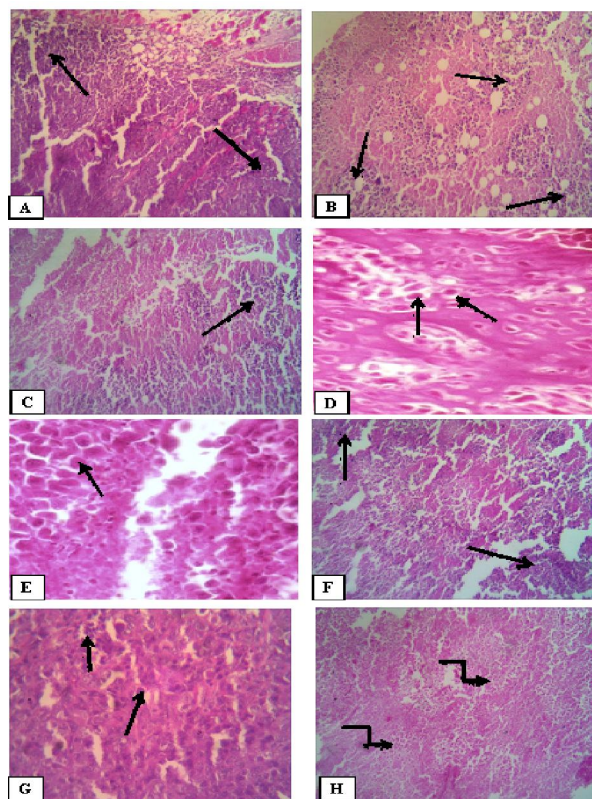


Fig.5. Histopathology of Ehrlich tumor cells with magnification 400x. (A) Photomicrographs of sections in Ehrlich solid tumor (EST) stained by H. and E for control group (neither laser nor drug treatment) Fig.5A: Section in Ehrlich solid tumor (EST). Fig.5B: Section in EST treated by photosensitizer. Fig.5 C, D and E x100: Section in EST exposed to laser radiation at different frequencies 1000,2000 and 3000Hz respectively in the absence of photosensitizer. Fig. 5F, G x100: Section in EST exposed to laser radiation at different frequencies 1000,2000 and 3000Hz respectively in the presence of photosensitizer.

Histopathological examination of the Ehrlich solid tumor (EST) under light microscope (Fig.5) with magnification x100. Showed compact and aggregation

of the tumor tissue cells spread within the muscular tissues (↑). In case of group Fig.5 (B) where tumors were injected with drug without any treatment with laser. In case of sham group (Fig.5(C, D and E) where tumors were exposed to laser with frequency 1000, 2000 and 3000 Hz respectively without drug injection, show viable islands of tumor cells and there is an increase in necrotic. Fig.5F, G and I x100: Section in EST exposed to laser radiation at different frequencies 1000,2000 and 3000Hz respectively in the presence of photosensitizer which shows tumor tissue sections represented high and wide zones of apoptotic cells and other many zones of tumor cells (Fig.5F), pleomorphism, hyperchromatism (Fig.5G), and totally necrotized for tumor (Fig.5H) for groups exposed to 3000Hz of laser radiation after drug injection which give us good improvement.

4. Discussions

Cancer is a class of diseases that increase year after year as it causes disorders due to uncontrolled cell division where uncontrolled cells are formed and the ability of these cells to invade other tissues either through the irregular growth of neighboring tissues through invasion or tumor of malignant tumor and other growth As a result of the damage caused to the DNA, leading to mutations in the genes responsible for controlling cell division. This results in a mutation in the DNA that may arise from chemicals, carcinogens or viruses. This mutation leads to the transformation of the healthy cell into a malignant cell.

Cancer can be treated by surgery, chemotherapy, radiation therapy, immunotherapy, monoclonal antibody therapy or other methods. The choice of therapy depends upon the location and grade of the tumour and the stage of the disease, as well as the general state of the patient. A number of experimental cancer treatments are also under development. Complete removal of the cancer without damage to the rest of the body is the goal of treatment.

Radiation therapy also called as radiotherapy, laser therapy, which is used to kill cancer cells and shrink tumor. Radiation therapy can be administered externally via external beam radiotherapy (EBRT) or internally via brachytherapy. Radiation therapy injures or destroys cells in the area being treated by damaging their genetic material, and enables to grow and divide. Photosensitizer is agents directly damage DNA to prevent the cancer cell from reproducing. As a class of drugs, these agents are not phase-specific and in other words, they work in all phases of the cell cycle⁽²⁸⁾.

Photodynamic therapy (PDT) is suggested by a number of researchers as a promising modality for the treatment of solid tumors and other non-malignant conditions. It comprises the use of a photo sensitizer

drug to enhance the absorption of the incident irradiation. It avoids many of the side effects of chemo and/or radiation therapy in that it is invasive modality since the photo sensitizer drug used in addition to exposure to light are not toxic by themselves. Also, in this promising modality, it is possible to irradiate only the tumor with a minimum or completely damage to the adjacent normal tissues) (28-29).

Figure 2 describes a tumor growth curve which is approximately an ascending curve (line) starting from the day of injection of cancer cells in normal mice to reach a maximum value on day 14.

During the fourteen days following the exposure of the tumor bearing mice to three different frequencies of low intensity laser therapy which exhibit a depression in the tumor volume that showing a plateau with the 1000 Hz, followed by that of the 2000 Hz, and ending with the 3000Hz infrared laser. So, it can be concluded that infrared laser has a depression effect on the tumor volume starting with day eleven with the 1000 and 2000 Hz, and with day nine with the 3000 Hz low intensity laser therapy.

It is clear that exposure to mice carrying the tumor to the infrared laser alone or in combination with the photosensitizer drug has a profound effect on tumor size with maximum effect in the presence of drugs and with maximum energy (or frequency), with 3000 Hz. However, from the pathological anatomy of the Erlich tumor using 3000Hz of lasers in the presence of light photosensitizer drugs has the most effect photosensitizer drug use has the most effect than using the photosensitizer drug alone and exhibit totally necrotized tumor. It could be concluded that the use of the photosensitizer drug alone has no effect on the Ehrlich tumor. However, the presence of the drug gave maximum effects with exposure to 3000 Hz infrared laser irradiation. This work requires an effort to reach more powerful effects using phototherapy with exposure to other different energies of the lasers.

5. Conclusions

From the data of this work, it can be concluded that; The photosensitizer drug alone has no effect on Ehrlich tumor. The use of infrared laser in the frequency range (1000 – 3000) Hz has an effect on tumor which is judged by the inhibition ratio of the tumor ($\approx 13.87\%$) on using 3000 Hz laser. Radiation-The infrared laser with frequency 3000 Hz in the presence of the drug is more effective than using infrared laser alone which give inhibition ratio of the tumor ($\approx 81.75\%$). The combination of exposure to infrared laser (3000Hz) in the presence of photosensitizer is more effective than infrared laser alone.

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