

Quantitative Study of the Evaluation of the Activity of Immune Cells in the Spleen of Diseased Mice with Cancer by Soft Laser

Aedah Z. Al-Kaisy Ahmed Anwar Albir

Department of Basic Sciences, College of Dentistry, University of Baghdad

Abstract

Background: Little informations have been published about the active role of soft laser in stimulation the immune cells, so, our work was conducted to examine experimentally the capacity of the immune cells such as macrophages of the spleen in reducing the tumor size of diseased mice with mammary gland carcinoma by soft laser. **Materials and Methods:** Fourty healthy female Swiss albino mice aged two months (weighing 45-48g) were used in the present experimental work. They were divided into five groups of six mice each. All the animals inoculated with mammary gland carcinoma. After successful inoculation, the animals were anaesthetized and irradiated with laser for 25 minutes with different time intervals and for ten days. The tumor size was measured before and after irradiation by vernier, daily, starting from tumor appearance until the last day of the experiment (10days). Sections of spleen were prepared and examined for histopathological study by light microscope. **Results:** The present experimental work showed reduction in tumor size of diseased mice with carcinoma irradiated with laser with the increasing of time interval for each experimental group of mice when compared to the tumor size of diseased mice with carcinoma not irradiated with laser. The histological examinations of the sections of the spleen of the diseased mice with carcinoma irradiated with laser showed abundant histological alterations in the structure of the macrophages such as increased size and multiplication of the nucleus when compared to the macrophages of the spleen of the diseased mice with carcinoma not irradiated with laser. **Conclusion:** The present experimental work proved that soft laser played an important role in activation the immune cells such as macrophages of the spleen and then decreased the tumor size of diseased mice with carcinoma and caused marked histological alterations in the structure of the macrophage due to laser action.

Keywords: Soft laser, Laser in medicine.

Introduction

Macrophages are characterized by the ability of phagocytosis. According to this state of activity power, they have a wide spectrum of morphological features. Macrophages are present in the most organs and represent the mononuclear phagocyte system. They are also characterized by being long living may remain alive for several months in the tissues. They perform as defensive elements. They phagocytize the debris of cell, unusual material elements, neoplastic cells, bacteria, and heavy elements that make a way into the organism. They also play a role as antigens-presenting cells that contribute in the processes of partial digestion and introducing of antigen to other cells. When macrophages are excited by any type of foreign materials, they alter their morphological characters and metabolism. In this case, they may called active macrophage that gain characteristics not found in their inactivated state. Moreover, these active macrophages in spite of showing an increase in their ability for phagocytosis and intracellular digestion, show also added metabolic and lysosomal enzyme activity⁽¹⁾. In the case of tumors, a high numbers of macrophages are existed in the tumor site⁽²⁾. Macrophages perform an important physiological role in the development and function of several tissues beginning from the brain until the mammary gland⁽³⁾.

Low level laser therapy (LLLT) has been used successfully in biomedicine and some of the results are thought to be related to cell proliferation. The effects of LLLT on cell proliferation is debatable because studies have found both an increase and a decrease in proliferation of cell cultures. Cell culture is an excellent method to assess both effects and dose of treatment⁽⁴⁾. In both soft tissue and connective tissue injuries, LLLT can increase the final tensile strength of the healed tissue. By increasing the amount of collagen production/synthesis and by increasing the intra and inter-molecular hydrogen bonding in the collagen molecules, laser therapy contributes to improved tensile strength⁽⁵⁾. Low level laser therapy (LLLT) has proved to be effective in treating and repairing biologically damaged tissue and to reduce pain⁽⁶⁾. In dentistry, LLLT is effectively used to accelerate recovery in cases of recurrent aphthous stomatitis, oral mucositis, traumatic ulcers, herpetic lesions^(7, 8), and treatment of temporomandibular disorders⁽⁹⁾. Concerning bone tissue, LLLT has been applied in several clinical situations, such as orthodontic treatment, alveolar repair after tooth extraction, bone fracture healing, and osseointegration of dental implants as an adjuvant therapy^(10, 11, 12). The stimulatory effects of LLLT include the following: ^(13, 14, 15, 16) proliferation of macrophages, proliferation of lymphocytes, proliferation of fibroblasts, proliferation of endothelial cells, proliferation of keratinocytes, increased cell respiration/ ATP synthesis, release of growth factors and other cytokines and transformation of fibroblasts into myofibroblasts. The aim of the present investigation is to put the laser in focus as a tool in activation the immune cells such as macrophages of the spleen in order to overcome the cancerous cells in diseased mice.

Materials and Methods

Forty healthy female Swiss albino mice aged two months (weighing 45-48g) were used in the present investigation. They were divided into five groups of six mice each. All the animals inoculated with mammary gland carcinoma (the last ten mice were kept as control group not irradiated with laser). After successful inoculation (the tumor appearance), the animals were ready for the experiment. The animals under study were anaesthetized and irradiated with laser for 25 minutes and for ten days. Laser beam was directed towards the spleen of the animals under study (the distance between the object and the laser source was 1cm) with time interval of irradiation for each experimental group of mice as in the following:

1. Group A: Irradiated with laser, four times daily with time interval of one hour.
2. Group B: Irradiated with laser, four times daily with time interval of 1.30 hour.
3. Group C: Irradiated with laser, four times daily with time interval of two hours.
4. Group D: Irradiated with laser, four times daily with time interval of 2.30 hours.
5. Group E: Irradiated with laser, four times daily with time interval of three hours.
6. Group F: Not irradiated with laser, inoculated with mammary gland carcinoma and was kept as control group, selected two animals for comparative purposes with each experimental group of mice.

At the end of the experimentation time of irradiation, the animals were sacrificed and their spleen was rapidly obtained. Sections of spleen were prepared by using a routine procedure and examined for histopathological study by light microscope. Photographs were made at different magnifications.

The tumor size of the animals under study was measured before and after irradiation by vernier, daily, starting from tumor appearance until the last day of the experiment (10 days).

The laser type used in this experiment was Gallium Arsenide of 50mw power and of 50mw/cm² power density.

Results

Forty healthy adult female of Swiss albino mice aged two months (weighing 45- 48g) were included in this experimental investigation.

Our results showed significant differences between the tumor size in diseased mice with carcinoma not irradiated with laser starting from 2.0 - 2.2cm to 4.27- 4.4cm (Table 1) during the entire period of experimentation time which was ten days and the tumor size in diseased mice with carcinoma irradiated with laser starting from 1.89 - 2.0cm to 1.2 - 1.3cm (Table 2) during the entire period of experimentation time (ten days). It could be said that the tumor size began to decrease with the increasing of time interval for each experimental group of mice respectively when compared to the tumor size of diseased mice with carcinom not irradiated with laser (Tables 1 and 2).

The histological examination of the prepared sections of the spleen of the diseased mice with carcinoma irradiated with laser showed obvious histological alterations in the structure of the macrophages such as increased size and multiplication of the nucleus (Figure 2) when compared to the macrophages of the spleen of the diseased mice with carcinoma not irradiated with laser (Figure 1).

Table 1: Showing tumor size (cm) of diseased mice with carcinoma not irradiated with laser.

Groups	Tumor size before irradiation (cm)	The mean of the tumor size before irradiation (cm)
A	2.0 - 2.2	2.1
B	2.5 - 2.7	2.6
C	3.6 - 4.0	3.8
D	4.1 - 4.3	4.2
E	4.27 - 4.4	4.33

Table 2: Showing decrease in tumor size (cm) of diseased mice with carcinoma irradiated with an increase of time interval.

Groups	Time interval	Tumor size after irradiation (cm)	The mean of the tumor size after irradiation (cm)
A	1 hour	1.89 - 2.0	1.94
B	1.30 hour	1.6 - 2.3	1.95
C	2 hours	2.9 - 3.5	3.2
D	2.30 hours	2.4 - 2.6	2.5
E	3.0 hours	1.2 - 1.3	1.2
F	Not irradiated group for comparative purposes		

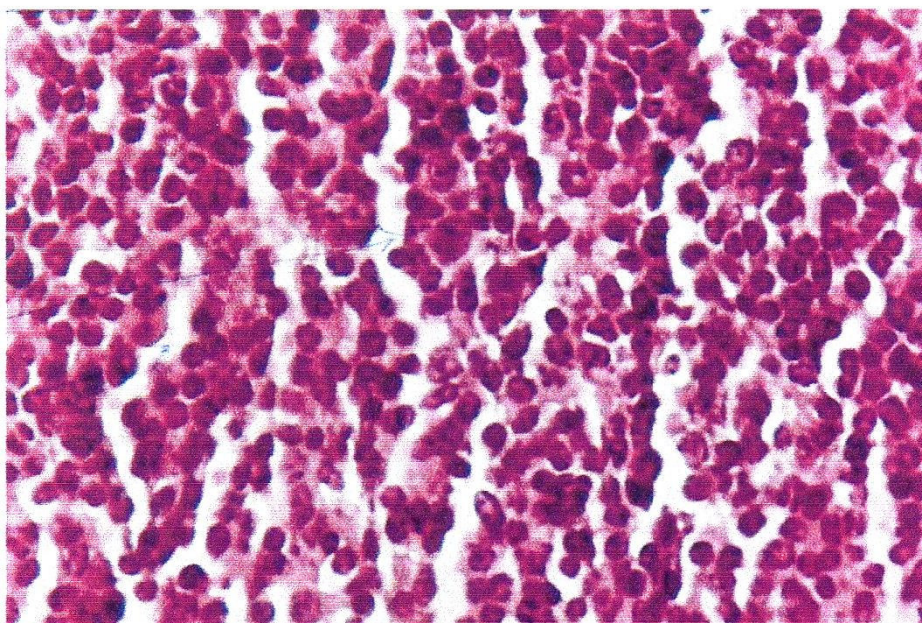


Figure 1. Macrophages of the mice spleen not irradiated with laser (H and E, $\times 40$).

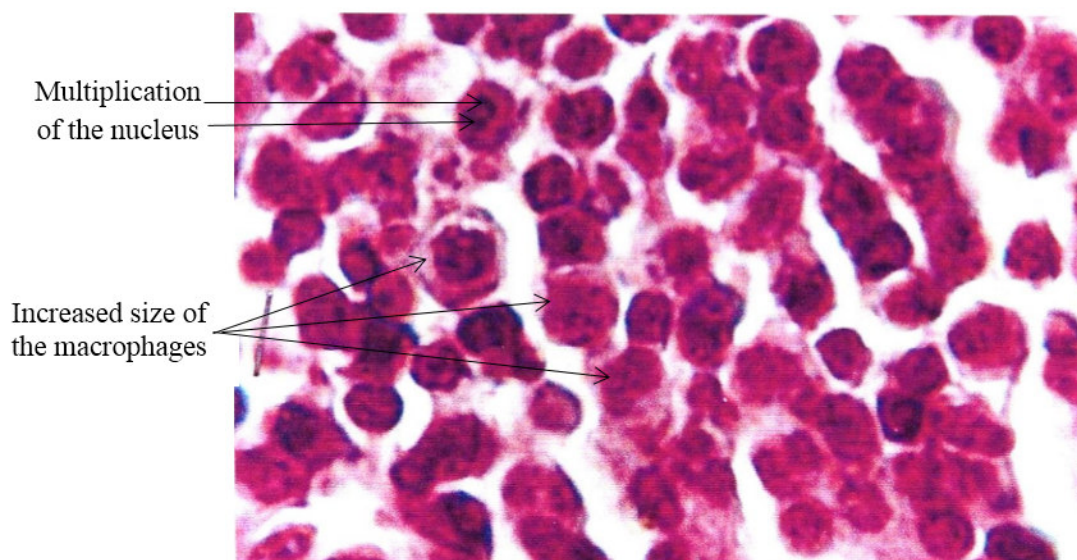


Figure 2. Macrophages of the mice spleen irradiated with laser showing obvious histological alterations such as increased size and multiplication of the nucleus (H and E, $\times 40$).

Discussion

The present investigation used the soft laser as a therapeutic tool to overcome the cancerous cells instead of drugs.

The main concentration of this experimental work was on the effect of the macrophages that found in the blood as antigen-presenting cells. The increasing in tumor size before irradiation of laser that occurred to the experimental groups of mice was due to the immune response against cancerous cells (Table 1). The decreasing of tumor size after irradiation with laser of the experimental groups of mice was proportional with the increasing of the time interval of irradiation (Table 2). The laser action caused the decrease in tumor size because as reported previously^(13, 14, 15, 16) that laser has stimulatory effects such as proliferation of macrophages which caused an increase in capacity of phagocytosis of these cells that resulted in an increase in the number of the cells in the location of the tumors which finally caused decrease in tumor size when compared to the tumor size of non-irradiated diseased mice. Also, it could be added that with the laser action, the immune ability increased and made macrophages activated and proliferate more quickly which caused abundant histological alterations in the structure of the macrophages such as increased size and multiplication of the nucleus (Figure 2) when compared to the macrophages of the spleen of the diseased mice with carcinoma not irradiated with laser (Figure 1).

Available literature indicates that no previous studies have been done to activate the immune cells such as macrophages by soft laser in order to overcome the cancerous cells and measuring the tumor size for comparative

purposes.

Finally, this phenomenon of soft laser can open a new era through adding a new knowledge to the treatment of cancer by stimulating the immune system that may attack the cancerous cells in order to inhibit its activity.

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