EURASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES
Innovative Academy Research Support Center
IF = 7.921
www.in-academy.uz

EVALUATION OF THE EFFECTIVENESS OF PHOTODYNAMIC THERAPY USING A NON-COHERENT LIGHT SOURCE WITH A WAVELENGTH OF 660 NM IN AN EXPERIMENT

Fozilova Sarvinoz Tursunboy Qizi
E-mail: vionadark84@gmail.com
Ozodbekova Sitora Alisher qizi
E-mail: Sozodbekova@gmail.com
Adilov Abdujabbor Abdukayumovich
E-mail: abdujabboradilov1@gmail.com
Saidmuratov Behruz Salaydin O`g`li
E-mail: Behruzsaidmurodov030@gmail.com
Kimyo International University in Tashkent, Uzbekistan
Musaeva Shahlo Najatovna
Scientific adviser:
E-mail: musayeva.shahlo.83@mail.ru
Kimyo International University in Tashkent, Uzbekistan
https://doi.org/10.5281/zenodo.16942003

ARTICLE INFO

Received: 18th August 2025
Accepted: 24th August 2025
Online: 25th August 2025

KEYWORDS

Photodynamic therapy (PDT), 5-aminolevulinic acid (ALA), fiber-optic light, cancer treatment, cytotoxicity, lymphocyte culture, Ehrlich tumor cells, red light irradiation, photosensitizer, in vitro model, cell viability, trypan blue assay, non-coherent light, phototoxic effect.

ABSTRACT

The increasing incidence of malignant neoplasms of various localizations is making them an increasingly serious problem for modern medicine, both in clinical practice and in prevention. Unfortunately, the proportion of advanced cases remains high, and tumor recurrences are often poorly responsive to repeated treatment and are accompanied by significant complications. This underscores the urgent need to improve methods for the diagnosis and treatment of cancer.

Photodynamic therapy (PDT) is a promising and rapidly developing approach to cancer treatment. Its progress directly depends on the development of specialized systems generating light in the 600–660 nm range. An important role is also played by both already known and new, more effective and accessible photosensitizers, as well as in-depth scientific studies aimed at revealing the mechanisms of the photodynamic effect. Evaluating the effectiveness of PDT is complicated by the fact that the photodynamic effect itself manifests minimally, and the death of tumor cells becomes evident only after a significant period of time.

In cancer treatment, photodynamic therapy (PDT) can be used both radically, with the goal of complete tumor destruction, and palliatively, to improve the patient's quality of life. This method is characterized by high selectivity, allowing healthy organs and tissues to be

preserved, as well as providing a good cosmetic effect. In addition, PDT can be performed repeatedly without the risk of serious local or systemic complications. Photochemotherapy represents another promising direction in the diagnosis and treatment of various types of cancer. This approach uses photosensitizers (external or internal), which, when activated by light, initiate chemical reactions in biological tissues

Research Objective:

To develop a laboratory (in vitro) model based on cell cultures for studying the effect of light exposure transmitted through fiber-optic systems in combination with a photosensitizer – a 10% solution of 5-aminolevulinic acid.

Materials and Methods

Due to the significant number of variables that can influence the course of photodynamic therapy (PDT), appropriate experimental models were selected. To assess the effectiveness of PDT, studies were conducted in two directions: in vivo and in vitro. Peripheral blood obtained from healthy individuals was used as a biological substrate, and isolated cells of Ehrlich's mouse ascitic tumor were also involved. A specialized device for photodynamic therapy was used to carry out the therapeutic exposure. The most important component determining the mechanism of action is the absorption and excitation spectrum of the photosensitizer used.

To create a lymphocyte culture, peripheral blood samples were taken from healthy volunteers. The culturing process was carried out using the whole blood method, following the technique developed by Arakaki D.T. and Sparkes R.S. in 1963. The main principle of this method is as follows: lymphocytes in peripheral blood culture are activated by phytohemagglutinin (PHA) – a purified mitogen derived from beans. Under the influence of PHA, lymphocytes begin to actively divide (enter the mitotic cycle) each day. The greatest number of dividing cells (mitoses) is observed 72 hours after the start of culturing. After this stage, the cells were incubated with the photosensitizer, 5-aminolevulinic acid, at a temperature of 37°C for three hours. They were then irradiated with a non-coherent light source with a wavelength of 660 nm for 30 minutes. At the same time, isolated cells of Ehrlich's mouse ascitic tumor were irradiated for 20 minutes.

To determine the quantitative indicator of cytotoxicity, the cells were stained with trypan blue. Then, using light microscopy with 400x magnification, the number of stained (i.e., dead) cells was counted. For this, the cell suspension was applied as a thick drop onto a microscope slide and mixed with an equal volume of a 0.1% trypan blue solution.

The value of cytotoxic activity (CTA) was determined using the following formula:

$$\mathbf{CTA\ (\%)} = \frac{A}{B} \times 100,$$

Where

A – number of dead cells;

B – total number of examined cells.

Statistical data processing.

Statistical data are presented as M±m (where M – arithmetic mean; m – standard deviation). For statistical analysis, the software package Statistica 5.0 was used. Comparison of independent groups was carried out using the Mann–Whitney test (T-test), and for the analysis of relative indicators, Fisher's t-test was applied. Differences between groups were considered statistically significant at a probability level of $p < 0.05$.

Research results and their discussion.

In the course of the study, it was found that photodynamic therapy (PDT) causes cell death, including cancer cells, through direct phototoxic action. To implement this approach, a portable device using red radiation was developed. Its purpose is the destruction of malignant tumor cells as part of a comprehensive cancer treatment. The therapeutic effect is achieved by activating a photosensitized reaction that is triggered by photons of light from the optical emitter.

The initial study of the new laser device using fiber-optic technology was aimed at studying its effect on a lymphocyte cell culture from healthy volunteers. It was found that the simultaneous use of a photosensitizer (5-aminolevulinic acid) and irradiation with non-coherent light at a wavelength of 660 nm causes a pronounced cytotoxic reaction (56%). This effect was statistically significantly higher than with the separate application of the photosensitizer (25%) or light (20%), as well as compared to the control (18%).

Table 1. Exposure to a non-coherent light source with a wavelength of 660 nm on a culture of lymphocytes from healthy donors

Exposure	Number of viable cells	Number of dead cells	Cytotoxic effect (CTE), %
Control, n = 100	82	18	18 ± 3.86
Photosensitizer (PS), n = 100	75	25	25 ± 4.35
PS + light irradiation, n = 100	44	56	56 ± 5.0*
Light irradiation, n = 100	80	20	20 ± 4.02

***- p<0.05**

At the second stage, mouse Ehrlich ascites tumor cells were incubated for 3 hours with a photosensitizer (5-aminolevulinic acid) at 37°C. Then the cells were subjected to 20 minutes of irradiation with non-coherent light at a wavelength of 660 nm (see Table 2).

Table 2.Exposure to a non-coherent light source with a wavelength of 660 nm on isolated Ehrlich ascites tumor cells.

Exposure	Number of viable cells	Number of dead cells	Cytotoxic effect (CTE), %
Control, n = 100	79	21	21 ± 4.1
Photosensitizer (PS), n = 100	73	27	27 ± 4.5
PS + light irradiation, n = 100	38	62	62 ± 4.9*
Light irradiation, n = 100	84	16	16 ± 3.7

***- p<0.05**

During the analysis of the effect of non-coherent radiation with a wavelength of 660 nm and 5-aminolevulinic acid on Ehrlich ascites tumor cells, it was found that monotherapy with

each of these factors caused minimal toxic response. The combined use of the photosensitizer and irradiation resulted in a pronounced cytotoxic effect, reaching 62%.

In the next stage of the study, a model of an experimental Ehrlich sarcoma strain in mice was used. Three animals with implanted Ehrlich tumors were intraperitoneally injected with 0.5 ml of a 5-aminolevulinic acid solution (concentration of 30 μg/ml in sterile water for injection) 3 and 20 hours before the start of irradiation (see Tables 3 and 4)

Table 3. Exposure to a non-coherent light source with a wavelength of 660 nm on Ehrlich ascites tumor cells 3 hours after the administration of the photosensitizer.

Exposure	Number of viable cells	Number of dead cells	Cytotoxic effect (CTE), %
Control, n = 100	97	3	3 ± 1.7
PS + light irradiation, n = 300 cell/ 3 animals	101	199	66.3 ± 2.7*

***- p<0.05**

Table 4. Exposure to a non-coherent light source with a wavelength of 660 nm on Ehrlich ascites tumor cells 20 hours after the administration of the photosensitizer.

Exposure	Number of viable cells	Number of dead cells	Cytotoxic effect (CTE), %
Control, n = 100	96	4	4 ± 3.9
PS + light irradiation, n = 300 cell/ 3 animals	117	183	61.0 ± 2.8*

***- p<0.05**

It was found that exposure to a non-coherent light source (660 nm, 30 minutes) and 5-aminolevulinic acid had a significant toxic effect on Ehrlich ascites tumor cells, causing the death of 66.3% and 61.0% of the cells, respectively. The duration of the preliminary exposure to the photosensitizer (3 or 20 hours) did not affect the degree of toxicity.

Conclusions

1.The experiment on lymphocyte culture showed that monotherapy with the photosensitizer (5-aminolevulinic acid) or standalone exposure to a non-coherent light source with a wavelength of 660 nm caused minimal toxicity. Combined exposure to the photosensitizer and light resulted in 56% cytotoxicity.

2.The experiment with Ehrlich ascites tumor cells revealed that both the use of 5-aminolevulinic acid as a photosensitizer and irradiation with non-coherent light at 660 nm individually caused minimal toxic effects. However, combined exposure to the photosensitizer and light led to a cytotoxic effect of 62%.

3.The study demonstrated that 30-minute exposure to non-coherent light with a wavelength of 660 nm, in combination with the photosensitizer 5-aminolevulinic acid, caused significant toxicity (66.3% and 61.0%, respectively) in Ehrlich ascites tumor cells. This effect was observed regardless of whether the cells were incubated with the photosensitizer for 3 or 20 hours.

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