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Photodynamic Effect of Fotoditazin on the DNA and the Nuclei of Erythrocytes of *Brachydanio rerio*

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Abstract:

The study is aimed at identifying the morphological and cytogenetic changes in erythrocytes of Brachydanio rerio, arising under the influence of photodynamic effects that models laser modification of blood, with the use of Fotoditazin, a photosensitizer of the chlorine range, and red laser radiation.

(*Methods of Research)* For 15 days, juvenile fish Brachydanio rerio were kept in settled tap water after providing model of laser modification of the blood using Fotoditazin and exposure to laser radiation with the wavelength of 630 nm. The impact on the structure of DNA in the nuclei of red blood cells of Fotoditazin, laser radiation and combined effects of these factors on the changes in the fluorescence spectra of DNA and the color of fluorescence were studied.

(Generalization of results and their meaning). It had been shown that separate effects of laser or Fotoditazin did not cause changes in the fluorescence of nuclei of erythrocytes, when the preparations were studied with the use of fluorescent microscopy after staining with acridine orange. After the fish were exposed for 5 minutes to the Fotoditazin solution with the concentration of 3.0 mg/l, and irradiated with red laser for 3 minutes with the power of 50 j/cm^2, the fluorescence of the nuclei of red blood cells dimed, which might have indicated the emergence of ruptures in the DNA molecules. After 24 hours, appearance of blood cells with the core fluorescing in orange color was noted, indicating appearance of single-chain DNA's. After 48 hours, intensity of fluorescence of red blood cells' nuclei approached the reference, but nuclei polymorphism was noted, which maintained until the end of the experiment. The impact of singlet oxygen in the process of model laser modification of the blood of fish with Fotoditazin affected the emergence of cytopathological violations in erythrocytes, the percentage of which increased veraciously, as compared to the reference in case of individual use of either laser or Fotoditazin. During laser modification of blood with photosensitizers in humans, it is recommended to pay attention to the possibility of DNA structure disrupting and appearance of cytopathological disorders in cellular blood elements, which requires further study.

Keywords: Fotoditazin, laser radiation, fish erythrocytes' nuclei, acridine orange, nuclei fluorescence, changes in the DNA structure, nuclei polymorphism.

INTRODUCTION

In recent years, the method of treating malignant tumors, i.e. photodynamic therapy (PDT) with the use of laser irradiation after introduction of photosensitizer [1], is being increasingly adopted.

In addition, laser modification of blood, which in its essence is similar to PDT, is used as a preventive measure, and as a measure for enhancing immunity of the organism. Photosensitizer is injected into the patient, then intravenous (IVLI) or above-venous laser irradiation of blood (AVLI) occurs [2, 3]. It is believed that laser modification of blood with a photosensitizer is more efficient than IVLI or AVLI, and some oncologists use it for patients with malignant tumors, and during pregnancy [4]. However, it is known that under laser activation photosensitizers promote emission of singlet oxygen, which acts as a mutagen, and causes disruption of DNA structure. DNA threads become fragmented, and single-chain DNA threads appear [5]. In this case, it is necessary to assess whether laser modification of blood causes such irreversible changes in the DNA of the nuclei of blood cellular elements, which may result in either formation of malignant cells or destruction of red blood cells.

We propose to assess the effect of laser blood modification on laboratory fish *Brachydanio rerio*, which is widely used in cytology and cancer research in many laboratories of the world [6 -8] and, compared to mammals, has the advantage of erythrocytes with nuclei with high content of DNA.

This study is aimed at identifying the morphological and cytogenetic changes in erythrocytes of Brachydanio rerio, arising under the influence of photodynamic effects that models laser modification of blood, with the use of Fotoditazin, a photosensitizer of the chlorine range, and red laser radiation. During the work, this goal has been achieved.

MATERIALS AND METHODS

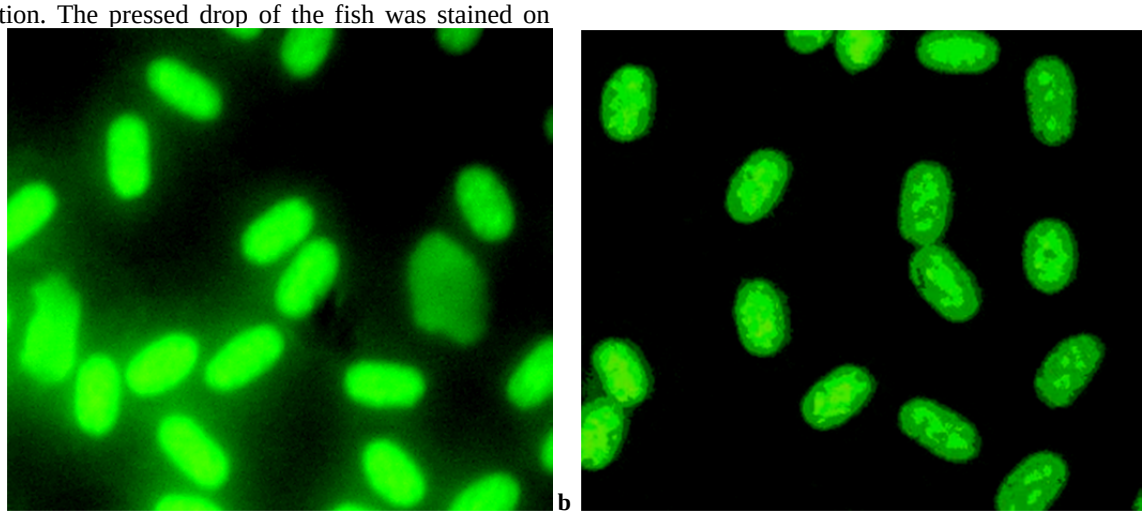
2 months old fish (Brachydanio rerio) with the length of 2 cm were placed into plastic aquariums (1 liter capacity) with settled tap water, 10 bions in each (reference). For the experiment, three batches of fish in aquariums of the same capacity were used. Experimentally, and by calculation, the parameters of the experiment were chosen that would approach those used in laser modification of humans. The fish in the first batch passed all stages of laser modification. They were kept in solutions of Fotoditazin photosensitizer with the concentration of 3.0 mg/l for 5 minutes, and after replacing Fotoditazin with water and placing fish into a glass with the area of the bottom of 7 cm^2, which corresponded to the area of the light spot, were irradiated with red laser "Milon" (wavelength 630 nm) for 3 minutes. The power density of irradiation was 50 j/cm^2. In the second batch of aquariums, fish stayed in settled water and received only laser irradiation with the same parameters, without the use of Fotoditazin. The third batch of fish remained only in the solution Fotoditazin, with the same exposure time, as the first batch. The experiment was repeated three times.

The fish were fed with granulated food "Esturgeon" with the grain size of 200-300 microns. Duration of the experiment was 15 days. Blood products were obtained from the severed tail-stem of fish after decapitation. The pressed drop of the fish was stained on the slide in two ways. To identify the state of DNA and RNA with the use of fluorescent microscopy, intravital staining of cell blood elements with acridine orange was used. Fresh drops of fish blood were stained according to the standard procedure [9] (Bertalanffy and Bickins) with 0.1% solution of acridine orange diluted with 9 parts of buffered Krebs-Ringer's solution. This method allowed monitoring DNA structure in the nuclei of erythrocytes and leukocytes as DNA denaturation and formation of its single-chain polymers in case of using acridine orange resulted in red or orange fluorescence, and a two-chain DNA in the complex with this dye resulted in green fluorescence. In addition, DNA ruptures caused by the influence of mutagenic agents resulted in dimming of luminescence, which also allowed monitoring changes in the structure of nucleic acid under the influence of unfavorable factors [10]. Preparations for fluorescent cytochemical analysis of fish blood were obtained one hour, 24 hours, and 48 hours after irradiation with laser. For morphological analysis of erythrocytes and leukocytes, and for the micronucleus test, smears were stained according to Romanovsky with azure blue [11] at the end of the experiment. The occurrence rate of erythrocytes and their nuclei anomalies was identified with the use of conventional methods [12-15]. Anomalies of erythrocytes and micronuclei (MN) were detected upon reaching 2,000 cells in preparations.

Fish erythrocytes were morphologically analyzed with pressed drop method in the field of view of microscope Nikon Eclipse 80i with the use of interference and phase contrast.

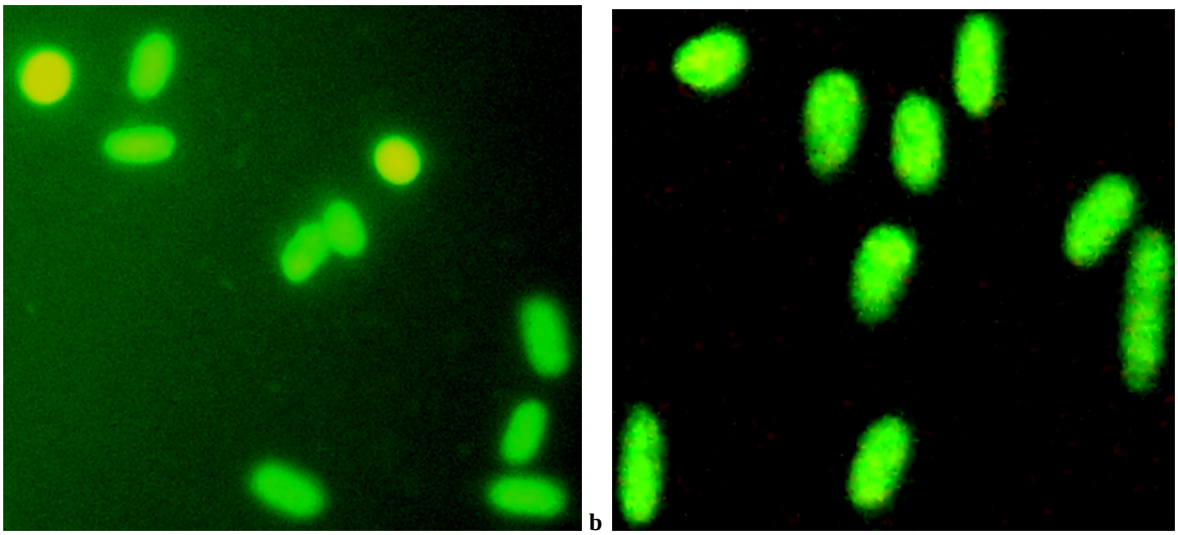
The innovation of this method lies in the fact that its application in the study provides an opportunity to observe changes in the structure of DNA of the finally differentiated cell (erythrocyte nucleus) by changes in the luminescence spectrum of the DNA & acridine orange complex after laser modification with Fotoditazin.

Significance of the difference of average indicators was assessed with the Student's t-test [16].



b

Figure 1. Fluorescence of erythrocytes' nuclei after intravital staining with acridine orange (seen in a fluorescent microscope, magnification 15 x 40). a - reference; b - one hour after model modification of blood (Fotoditazin and laser irradiation) (cytoplasm of erythrocytes is not visible, fluorescence is dimed by hemoglobin).



b

Figure 2. Fluorescence of the nuclei of erythrocytes and lymphocytes after various periods of keeping fish in solutions of Fotoditazin and laser irradiation:
a - 24 hours after experimental modification of blood; b - 48 hours after exposure to Fotoditazin and laser irradiation.

RESULTS

Fluorescent analysis of DNA molecules in erythrocytes of zebrafish in three above-mentioned variants of the experiment showed the following. The use of only Fotoditazin with subsequent keeping zebrafish in a shaded room, or only laser radiation, did not change the fluorescence of the nuclei of blood cellular elements stained with acridine orange. In all studied periods after using laser or keeping fish in solutions of Fotoditazin, DNA in the nucleus fluoresced bright green, the same as in the reference.

An absolutely different picture was observed in case of combined action of photosensitizer and laser radiation. Blood of reference and experimental fish is shown in Figure 1 (a, b).

The study had shown that keeping fish in Fotoditazin solution with the concentration of 3.0 mg/l and the following exposure to red laser resulted in partial dimming of luminescence of the DNA & acridine orange complex in the nuclei of erythrocytes, which indicated disruption in the structure of DNA (most likely, gaps between the threads). In addition, separate areas of increased fluorescence were visible in the nuclei, which might have indicated intact DNA structure in these areas. Thus, direct influence of the photosensitizer on the genetic apparatus of the cell under laser irradiation could be determined.

Further monitoring of the DNA state in the nuclei of erythrocytes showed that the effect of dimming luminescence continued for 24 hours, which indicated breakage of DNA threads after model modification of blood. However, uniform luminescence of nuclei was observed, which allowed speaking of further rebuilding of DNA molecules. Along with this, in young erythrocytes with round nucleus (on the right in Figure 2 a), and in the nuclei of lymphocytes (on the left in Figure 2 a) orange glow was visible, which indicated further rebuilding of the DNA and emergence of the difference in DNA chains, which might result in the death of the cell. Figure 2 b shows fluorescence of erythrocytes' nuclei two days after experimental modification of fish blood with the use of Fotoditazin, which corresponds by its intensity to the reference group; polymorphism of the nuclei is also observed, which is preserved after staining blood preparations according to Romanovsky upon completion of the experiment.

Since the morphological changes in blood cells of fish were preserved until the end of the experiment, micronuclei and pathologic cytological disruptions after photodynamic treatment were counted at the end of the experiment, which took 15 days.

The occurrence rate of erythrocytes with micronuclei in the reference bions was quite significant, and was equal to 1.1 %.

In case of the fish exposure only to red laser for 3 minutes, no sharp increase in the number of micronuclei was observed in erythrocytes of fish, compared to the reference group; this influence might be considered veracious.

During the study of erythrocytes in fish kept in Fotoditazin solutions for 5 minutes followed by keeping in a dark room for 15 days, a slight increase in the number of micronuclei (up 1.8%) was observed, which indicated very weak mutagenic properties of the chlorine photosensitizer. Upon combined action of the photosensitizer and laser radiation, when the photodynamic effect was detected, the number of micronuclei increased to 2.5 %, which also indicated weak mutagenic effect of the photodynamic process. However, in this case, the greatest morphological changes in the nuclei of fish erythrocytes were observed, which was probably caused by the release of singlet oxygen during the photodynamic treatment. At the same time, polymorphism of nuclei increased, which had already been mentioned before (one of the symptoms of the toxic effect) (See Table 1).

Table 1. Types of morphological disruptions (%) in the nuclei of erythrocytes of Brachydanio rerio and their occurrence rate 15 days after the postphotodynamic effect with the use of Fotoditazin

Main types of disruptions	Reference	Laser irradiation	Fotoditazin	Fotoditazin and laser
Micronuclei (MN)	1.1	1.3	1.8*	2.5*
Nuclei displacement to the periphery	3.9	4.4	8.2*	14.0*
Deformation of nuclei	10.3	12.6	12.6	24.6*
Ring nuclei	0.0	0.5	0.0	1.9
Protuberances	6.0	6.6	7.5	19.8*
The number of examined erythrocytes	2,000	2,000	2,000	2,000

Note: - credibility of the difference in the average values between the reference and experimental groups according to the Student's t-test ($P \leq 0.05$).*

DISCUSSION

Increased expression of pathological forms of the erythrocytes' nuclei was detected (see Table) in the peripheral blood of zebrafish in the course of the photodynamic reaction. Similar phenomena are usually caused by phototoxicants. This work is characterized by the fact that the action of Fotoditazin and laser radiation has been studied on the nuclei of fish erythrocytes, where most genes in the DNA are repressed. Therefore, it can be expected that laser modification of blood may have impact on the nuclear material of other terminally differentiated cellular elements of blood. It is possible to suggest that it may be mediated via changes in protein expression as reported before [17, 18].

The data in the table show that in the course of photodynamic treatment that models laser modification of blood, the occurrence rate of some cytopathological indicators in the nuclei of erythrocytes in the studied population of fish increases, and DNA structure gets disrupted in the nuclei of erythrocytes of fish. During the first day after laser modification blotchy areas are visible in the nuclei, where DNA fluorescence is dimmed, indicating transverse ruptures in DNA threads. Next, DNA reparation occurs, and orange fluorescence appears, which characterizes discrepancies in the DNA chains; however, they disappear two days later. On the other hand, the number of deformed nuclei increases, protuberances and micronuclei appear. Apparently, with the change in the DNA structure under the influence of the studied factors, nuclei of erythrocytes with morphological abnormalities appear, and the occurrence rate of micronuclei increases.

Given the close similarity of DNA of any organism's response to the effects of reactive oxygen forms, it can be assumed that laser irradiation of blood with the use of photosensitizers, except for photodynamic therapy (PDT), will have influence on the human organism, especially on the one suffering from cancer. Further study of this issue is required, all the more so that the person is already undergoing laser modification of blood during PDT.

CONCLUSION

Thus, as a result of work with laboratory object Brachydanio rerio, it has been shown that modeling of laser modification of blood with Fotoditazin may result in changes in the structure of DNA in erythrocyte nuclei and in manifestation of cytopathological forms in them. The regularity has been found that the number of abnormal erythrocyte nuclei increases after laser modification of blood even when reversible disruptions occur in the DNA, and DNA is capable of restoring itself. Despite the fact that low mutagenic activity of Fotoditazin after exposure to laser has been established as a result of the work, the process of laser blood modification should be approached with caution, especially in patients with cancer, who have depressed immune system. Certainly, the mutagenic activity of chlorine preparations should be studied further; it is important to answer the main question, i.e. whether the use of Fotoditazin and other photosensitizers is justified for laser blood modification, which requires a number of sessions, which, in turn, may cause structural changes in the DNA.

REFERENCES

1. Kaplan, M. A., Kapinus, V. N. and Popuchiev, V. V. et al. Fotodinamicheskaya terapiya rezultaty i perspektivi. [Photodynamic therapy: results and perspectives]. Radiation and risk, 2013, 22(3): 115-119.
2. Chernova N. I., Perlamutrov Y. N., Olkhovskaya S. B. and Moskvin S. B. Vnutrivennoe lazernoe obluchenie krovi v lechenii tsitomegalovirusnoi infektsii urogenitalnogo trakta seksualno aktivnyh zhenschin [Intravenous laser blood irradiation in the treatment of cytomegaloviral infection of the urogenital tract in sexually active women]. Physical therapy and rehabilitation, 2013, 5: 19-24.
3. Lapchenko A. S., Kucherov A. G. and Levina, Y.V., et al. Above vessel laser irradiation of blood in treatment of cochleovestibular disorders. Medical care, 2011, 3: 75 – 77.
4. Hashukeva A. Z., Svetich O. A. and Markova E. L. et al. Effektivnost fotosensibilizirovannoi modifikatsii krovi v lechenii gerpesvirusnoi infektsii pri privichnoi nedonashivaemosti beremennosti [Efficiency of the photosensibilized modifications of blood in treatment of herpesvirus infections in case of habitual abortive pregnancy]. Bulletin of the Russian State Medical University, 2014, 4: 27 – 32.
5. Martusevich A. A., Peretyagin S. P. and Martusevich A. L. Molekulyarnie i kletochnie mehanizmi deistviya singletnogo kisloroda na biosistemi [Molecular and cellular mechanisms of singlet oxygen action on the biological systems]. Modern technologies in medicine, 2012, 2: 128 -131.
6. Traver D. and Winterer A, et all., Effects of lethal irradiation in zebrafish and rescue by hematopoietic cell transplantation. Blood 1, 2004, 04: 1298–1305.

7. Niethammer Ph. and Clemens A., et all. A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. Cell Death Differ, 2009 1: 431-442.
8. Oren M. Decision making by p53. Life, death and cancer, 2003, 3: 245 – 248.
9. Pierce E. Gistohimiya [Histochemistry]. Mosxow: IL, 1962, pp. 944.
10. Karnaukhov V. N. Lyuministsentnii analiz kletok. [Luminescent analysis of cells]. Puschino, 2002, pp. 120.
11. Romeis B. Mikroskopicheskaya tekhnika [Microscopic methods]. Moscow: Foreign literature, 1954, pp. 718.
12. Hasanov S. H. and Simakov, Y.G. Biotestirovanie genotoksichnosti zagryaznitelei vodi po obrazovaniyu mikroyader v eritrotsitah danio. Biodiagnostika v ekologicheskoi otsenke pochv i sopredelnih sred. [Biological testing of genetic toxicity of water pollutants by formation of micronuclei in erythrocytes of zebrafish. Biological diagnostics in ecological assessment of soils and adjacent media]. Abstracts of the international conference. Moscow. February 4 -6, 2013. Moscow: BINOM, 2013, pp. 42.
13. Lilly R. Patogistologicheskaya tehnika i prakticheskaya gistohimiya [Histopathological method and practical histochemistry]. Moscow: Mir, 1969, pp. 645.
14. Ivanova N. T. Atlas kletok krovi rib [Atlas of blood cells of fish]. Moscow: Light and food industry, 1983, pp. 184
15. Zhiteneva L. D. Atlas normalnyh i patologicheski izmenennyh kletok krovi rib [Atlas of normal and pathologically changed cells of fish blood]. Rostov-on-Don: Editing House, 1989, pp. 112.
16. Lakin G. F. Biometriya [Biometrics]. Moscow: Vysshaya Schkola, 1973, pp. 343.
17. Trushin, M.V. The effect of red and infrared light on the growth of Escherichia coli and the production of the recombinant protein barstar. Microbiology2002; 71(4): 383-385.
18. Trushin, M.V., Yagodina, L.O., Chernov, V.M. The modification of biostimulating effect of IR laser radiation by genetic transformation. Laser Physics 2002; 12(4): 656-658.