Research Article

Study of the Effect of the Photosensitizer Psoralen on the Exudative and Proliferative Stages of Inflammation

Iriskulov Bakhtiyar, Abdukhalikova Nigora, Rakhmonov Alisher, Makhmudova Feruzabonu, Ubaydullayev Elbek

Department of Normal and Pathological Physiology, Tashkent Medical Academy, Tashkent, Uzbekistan

Corresponding author:
Abdulhalikova Nigora,
Department of Normal and Pathological Physiology,
Tashkent Medical Academy,
Tashkent, Uzbekistan,
E-mail: strageticmanagement21@gmail.com

Abstract

The aim of this study is to study the effect of various schemes (with and without ultraviolet irradiation) of the use of the photosensitizer-psoralen in exudative and proliferative stages of inflammation, as well as the effect on the cellular composition of peripheral blood and on biochemical parameters. Male rats were used in the experiment. It was shown that, under these conditions, the use of psoralen + UFO was superior to the use of psoralen itself. This combination significantly inhibited the proliferation and exudation phase in aseptic inflammation model. The data obtained expand and deepen the understanding of the use of plant-derived photosensitizers in inflammatory processes in a clinical setting.

Keywords: Photodynamic therapy; Photosensitizers; Psoralen; Reactive oxygen species; Mitochondrial pore

Introduction

Photodynamic Therapy (PDT) is one of the promising areas of modern photobiology and in recent years has been undergoing rapid development in connection with new developments in the field of diagnosis, prevention and treatment of human diseases.

PDT is based on the introduction into the body of chemical preparations-Photosensitizers (PS), which have an increased tropism for target cells (cancer cells, inflammatory tissues, microbes and viruses). [1-3] Under the influence of light of a certain wavelength and energy, PS begin to produce atomic (singlet) oxygen, as well as generate other Reactive Oxygen Species (ROS), which cause oxidative damage to various molecules (proteins, unsaturated fatty acids, nucleic acids) and cell structures (membranes, enzyme systems, genetic apparatus, etc.), which entails the inactivation of pathogens. [4]

For photodynamic therapy, photosensitizers are used that differ significantly in their physicochemical and photo physical properties, and the conditions for their photo activation vary considerably. [5,6]

A drug for PDT must have chemical purity and uniformity of composition, be free of toxicity, have a high capacity for accumulation in the target tissue, rapid elimination from the patient's body, high photochemical activity, which is characterized by a high quantum yield of singlet oxygen and absorption of light in the long-wavelength part of the spectrum (600 nm-800 nm), the so-called "therapeutic window". Under these conditions, where the intrinsic absorption of biological tissue is minimal, a deeper penetration of radiation into the tissue is ensured and, as a consequence, a high efficiency of therapy. In recent decades, dozens of substances with photosensitizing properties have been synthesized. Although the "ideal" photosensitizer has not yet been obtained, preparations have been developed that approach the required characteristics in a number of properties.

Medicinal plants play an important role in the production of medicinal products. The value of natural Biologically Active Substances (BAS) of plant origin is associated with the fact that their chemical nature is close to the human body and is easily included in biochemical processes. As a rule, biologically active substances have a wide spectrum of biological activity and many of them have low toxicity. [7] They are less likely to cause allergic reactions. In addition, the advantage of herbal medicines is that in the treatment of chronic diseases, they do not cause any side effects with prolonged use. However, unlike most chemotherapeutic agents, the mechanism of their pharmacological activity has not been sufficiently studied and their use is based mainly on many years of experience in traditional medicine.

Studies of employees of the institute of chemistry of plant substances of the academy of sciences of the republic of Uzbekistan have established that a number of plants of the flora of Uzbekistan are sources of natural compounds with photodynamic properties. Fig is one such plant with photosensitizing activity. Scientists have found that fig leaves contain significant amounts of psoralen and bergapten, two of the most active furocoumarins. [8] Under the influence of light, psoralen is able to modify biological molecules in two ways: as a result of oxygen independent photo addition reactions to unsaturated organic molecules (primarily to DNA) and due to oxidative photoreactions. [9]

In clinical practice, inflammation is the leading patho genetic link in many diseases (oncological, neurodegenerative, endocrine). The proportion of chronic forms of inflammatory diseases is quite high (from 56% to 78% of all pathologies, the genesis of which is inflammation). [10,11] An extreme degree of chronic inflammation is granulomatous inflammation. It is

How to Cite this Article: Bakhtiyar I, et al. Study of the Effect of the Photosensitizer Psoralen on the Exudative and Proliferative Stages of Inlammation. Ann Med Health Sci Res. 2022;12:2:39-43.

characterized by a wide range of changes due to a long delay in the tissues of the damaging factor. [12,13] A clear characterization of various types of cells and mediators involved in the dynamics of inflammation is extremely important for a targeted search for drugs that affect certain stages of it.

All of the above determined the goal of this study-to study the effect of various schemes (with and without ultraviolet irradiation) of the use of psoralen in the exudative and proliferative stages of inflammation.

Materials and Methods

The experiments were carried out on sexually mature white male rats with an initial weight of 150 g-225 g, which were kept under standard vivarium conditions, quarantined for at least 12-14 days. The study of anti-inflammatory activity was carried out using the "felt granuloma" method. [14] For the study, 5 groups were formed from clinically healthy rats with clean skin.

1-2 days before the experiment, the hair was carefully cut in the back area and a 1 cm long incision was made in the skin and subcutaneous tissue under aseptic conditions. 1-2 sutures were applied. The animals of the third and fourth groups were injected intra gastrically, respectively, the drug psoralen-10 mg/kg, on the day of surgery and for the next seven days every three days. After 2 hours, the fourth and fifth groups were irradiated with UV rays ("quartz-8" lamp with DRT-1000 with a power of 220 watts, manufactured by "Magnum medical servis" LLC, RUz). On the day of surgery, the animals were irradiated for 2 minutes. Day 4-2 minutes 30 seconds, day 7-3 minutes. [15] The initial radiation dose was selected by phototesting with the determination of the Minimum Erythemal Dose (MED). It was found that the initial radiation dose is 2 minutes.

A day after the last injection of drugs (on the eighth day), after taking blood from the tail vein for hematological and biochemical studies, the animals were sacrificed by one-stage decapitation under light ether anesthesia. The cotton balls with the granulation tissue formed around them were removed, weighed on an electronic balance (SINKO, Japan) and dried at a temperature of 60°C for several days to constant weight. The degree of the proliferative phase was judged by the difference between the mass of the formed granulation-fibrous tissue of the dried granuloma and the initial mass of the ball. The exudative reaction was assessed by the difference between the weights of the raw and dried granulomas. [16]

The experiments were carried out in accordance with the "Rules for conducting work with the use of experimental animals", as well as with the rules of the European convention for the protection of animals used for experimental research or for other scientific purposes (ETS No. 123, Strasbourg, 18.03.1986).

The obtained results of experimental studies were processed by the method of variation statistics using the standard software package stat plus 2009 with an assessment of the significance of indicators (M ± m) and differences in the samples under consideration by the student's t-test.

Results and Discussion

Evaluation of anti-inflammatory activity showed that the development of granulomas was inhibited by all investigated procedures. When using psoralen 10 mg/kg without irradiation, the mass of dry granulation fibrous tissue was 0.042 g ± 0.003 g, which was 60.3% lower than the control values. So, the mass of exudate was 0.177 g ± 0.02 g, which is 48% less than the same indicator of the control group of animals [Table 1]. This means that the effectiveness of the use of psoralen 10 mg/kg without irradiation was superior to the use of psoralen 10 mg/kg + UV. So, when using this combination, the mass of dry granulation fibrous tissue was 0.055 g ± 0.003 g, and the mass of exudate was 0.291 g ± 0.013 g, which is lower than the control values by 48.1% and 14.6%, respectively. And when using UV irradiation itself, the mass of dry granulation-fibrous tissue was 0.049 g ± 0.003 g, and the mass of exudate was 0.269 g ± 0.02 g. This is less than the indicators of the control group by 53.7% and 21%, respectively [Table 2].

Peripheral blood condition, hematological studies have shown that the tested drugs and treatment procedures lead to a significant change in the content of blood corpuscles in comparison with the control group. When observing the level of leukocytes in the peripheral blood of rats, a pronounced increase in leukocytes in the control group of 20.78 ± 1.5 was revealed, which is 43.4% higher than in the intact group. [17-28] The same sharp increase in leukocytes was observed in the fifth group, in which the animals received only ultraviolet irradiation: 25.33 ± 2.25, which is 74.8% higher compared to the intact group and even 21.8% higher in compared with the control group. However, in the groups that received the plant photosensitizer psoralen 10 mg/kg with and without irradiation (groups 3 and 4), the number of leukocytes significantly decreased: 17.22 ± 1.6 and 16.88 ± 0.8, which was lower control values by 17.1% and 18.7%, respectively [Table 3].

There was an increase in the total number of granulocytes in the fifth group of 14.27 ± 2.25, which is 71.5% higher than that of the control group of animals. This proves that UV irradiation without the use of a photosensitizer enhances the inflammatory

Table 1: Scheme of the experiment according to the "felt granuloma" technique on rats.

Group of animals	Used schemes	Time and distance of UV exposure 2 hours after taking the drug			Method and frequency of administration of psoralen
		On the day of surgery	4-day	7-day	
1-intact	-	-	-	-	-
2-control	0.9% sodium chloride solution	-	-	-	Intra gastric 1 time every 3 days for 7 days
3-experience	Psoralen 10 mg/kg	-	-	-	
4-experience	Psoralen 10 mg/kg+UV	2 minutes at a distance of 50 cm	2 minutes 30 seconds at a distance of 50 cm	3 minutes at a distance of 50 cm	
5-experience	UFO	2 minutes at a distance of 50 cm	2 minutes 30 seconds at a distance of 50 cm	3 minutes at a distance of 50 cm	-

Table 2: Influence of psoralen. UVR and psoralen+UVR on the stage of inflammation in rats n=6 (M ± m).

Observation group	Number of animals	Dry mass of granulation fibrous tissue. gr	Inhibition of proliferation %	Exudate mass gr	Inhibition of exudation %
Control	6	0.106 ± 0.004	-	0.341 ± 0.03	-
Psoralen 10 mg/kg	6	0.042 ± 0.003	60.3%	0.177 ± 0.02	48%
Psoralen 10 mg/kg+UV	6	0.055 ± 0.003	48.1%	0.291 ± 0.013	14.6%
UFO	6	0.049 ± 0.003	53.7%	0.269 ± 0.02	21%

Table 3: Hematological parameters of rats exposed to psoralen. psoralen+UFO and UFO.

Groups		Leukocytes. 10^9/l WBC	Absolute lymphocyte count. 10^9/l	The absolute content of a mixture of monocytes. basophils and eosonophils 10^9/l	Quantity granulocytes. 10^9/l	Platelets in absolute numbers. 10^9/l PLT	Thrombokrit. % PCT
1-group Intact	M ± m	14.49 ± 1.5	6.12 ± 0.5	2.08 ± 0.28	6.5 ± 0.5	604.3 ± 66.68	0.519 ± 0.05
2-group control	M ± m	20.78 ± 1.5	9.18 ± 0.45	2.8 ± 0.18	8.32 ± 1.11	656.7 ± 54.9	0.558 ± 0.035
3-group Psoralen	M ± m	17.22 ± 1.64	7.22 ± 0.4	1.72 ± 0.07	8.27 ± 1.55	875 ± 175.17	0.603 ± 0.103
4-group Psoralen+UFO	M ± m	16.88 ± 0.82	6.85 ± 0.47	2.67 ± 0.19	7.37 ± 0.57	721.5 ± 72.77	0.580 ± 0.04
5-group UFO	M ± m	25.33 ± 2.25	8.92 ± 0.53	2.15 ± 0.24	14.27 ± 2.25	762.17 ± 65.9	0.580 ± 0.04

Table 4: Biochemical parameters of rats exposed to psoralen. psoralen + UFO and UFO.

Groups		AlT	AST	Alkaline phosphatase	Gamma glutamyl transferase	Total protein
1-group Intact	M ± m	64.80 ± 2.56	208.83 ± 16.35	340.92 ± 63.05	5.83 ± 0.5	80.20 ± 4.67
2-group control	M ± m	52.32 ± 3.89	288.33 ± 13.82	465.67 ± 70.15	6.0 ± 0.58	112.25 ± 4.65
3-group Psoralen	M ± m	87.17 ± 2.96	301.17 ± 25.84	548.52 ± 72.72	6.71 ± 0.64	92.07 ± 7.57
4-group Psoralen+UFO	M ± m	56.00 ± 3.42	196.67 ± 13.79	326.0 ± 22.31	11.83 ± 0.49	100.18 ± 3.57
5-group UFO	M ± m	60.68 ± 4.70	249.50 ± 6.76	586.83 ± 32.51	9.00 ± 0.65	101.52 ± 4.56

process [Table 3]. During the experiments, it was found that in animals treated with psoralen 10 mg/kg+UV, there was a slight decrease in the total number of granulocytes 7.37 ± 0.57, which is 11.4% lower than the values in the control group.

A number of quantitative changes are also observed in platelets. In the experimental group, which received only psoralen 10 mg/kg, without irradiation, the number of platelets increased by 875 ± 175.17, which is 33.2% higher than that of the control group [Table 3].

The data of a comparative analysis of the results of biochemical studies of the blood of rats are given in Table 4. When choosing biochemical blood markers, we were guided by a standard set of indicators that allow us to characterize the functional state of the main organs and systems of the body. As a result of studies of blood samples, it was found that in rats of the control group, modeling of chronic inflammation causes characteristic biochemical changes. In animals treated with 10 mg/kg psoralen, the ALT concentration was 87.17 ± 2.96, which means an increase in this indicator by 66% compared with the control group. Similar changes were noted in the study of the concentration of gamma glutamyltransferase [Table 4]. In the fourth group, which was injected with psoralen 10 mg/kg and irradiated, the gamma glutamyl transferase index increased almost 2 times, 11.83 ± 0.49. And in the fifth group, the same indicator is 9.00 ± 0.65, that is, 50% higher than in the control group.

Conclusion

Today, for the treatment of various diseases PUFO-A-therapy (P-psoralen, UFO-A-ultraviolet irradiation, spectrum A) is widely used. Various researchers give an ambiguous picture of the effectiveness of this type of treatment [17,18]. That is why, in the experimental treatment of aseptic inflammation on the model of "felt granuloma", we used separately treatment with ultraviolet light, separately treatment with psoralen and the combined use of these two methods of treatment. The results of the effectiveness of the treatment regimens used for the proliferative and exudative activity of the studied tissues, obtained by us, are significantly higher in the combination of psoralen and ultraviolet irradiation. This is apparently due to the presence of the ability of psoralen to inhibit the generation of superoxide anions both in granulocytes and in cells of connective tissue elements [19, 20, 21, 22]. In combination with an increase in reparative synthesis in cells under the action of UVA-A, it leads to an increase in the anti-inflammatory effect of the agents used [19]. These and possibly other mechanisms of action of these influences predetermine the relatively high efficiency of the combined use.

In fairness, it should be noted that the combined use of psoralen and UFO gives the lowest percentage of liver complications. Psoralen can damage the liver through the formation of reactive furan epoxide, which leads to irreversible inhibition of cytochrome P450, the main enzymatic system of

biotransformation of xenobiotics in hepatocytes [23]. Psoralen can cause toxic liver damage by affecting a number of genes that cause hepatotoxicity [23,24]. When psoralen is used with UFO, psoralen significantly inhibits liver mPTP (mitochondrial permeability transition pore). This mitochondrial Ca2+ -dependent pore is formed by a complex of proteins and is a channel that passes through the outer and inner membranes of mitochondria. It is known that at the mPTP level many physiological processes of mitochondria and cells are regulated [25]. Increased permeability of mitochondrial membranes leads to the development of various pathologies: the formation of reactive oxygen species, dysfunction of ion channels, LPO activation, oxidation of membrane thiol groups, etc. [25, 26, 27]. Inhibition of this pore stabilized the mitochondrial membranes. The opening of mitochondrial membranes was also observed under the action of a separate application of ultraviolet radiation [28].

Based on the above, the combined use of psoralen and UFO is the most appropriate in terms of both enhancing the anti-inflammatory effect of photosensitization therapy and preventing possible side effects of psoralen. If it is necessary to use psoralen for a long time in therapy, it will be advisable to use hepatoprotectors of plant origin.

Acknowledgements

We would like to express our sincere gratitude to our assistant, Rakhmatullaev Elyorbek for technical designing and preparing manuscript

Conflict of Interest

The authors disclose that they have no conflicts of interest or competing interests. The authors state that the manuscript has been read and approved by all the authors, that the requirements for authorship as stated in the instructions to authors have been met, and that each author believes that the manuscript represents honest work.

References

1. Bril EA, Smirnova YV, Yashchuk VO, Reshetneva IT, Bril VI. Application of the photodynamic effect in the prevention of inflammatory periodontal diseases in orthodontic patients. Actual Probl Mod Sci Educ and Train. 2015;6.
2. Martusevich AK, Martusevich AA, Peretyagin SP. Molecular and cellular mechanisms of singlet oxygen action on biosystems. Modern Technologies in Medicine. 2012;2:128-134.
3. Hamblin MR. Mechanisms and applications of the anti-inflammatory effects of photobio modulation. AIMS Biophysics. 2017;4:337-361.
4. Korshunova OV, Plekhova NG. Photodynamic therapy in oncology: present and future. Pac Med J. 2020;4:15-19.
5. Uzdensky AB. Cellular and molecular mechanisms of photodynamic therapy. Science. 2010.
6. Rusakova OA, Ralchenko IV, Herbert IY, Verdieva SI. Study of the pharmaceutical assortment of phytopreparations. J Pharm Pharmacol. 2015;6:54-59.
7. Yarosh EA, Gogmachadze ID, Apakidze IA. The fruit culture of figs is a source of medicinal products. Agro Eko Info. 2008;1:10.
8. Laman NA, Kopylova NA. Natural furocoumarins as photosensitizers and promising components of drugs. Collection of Scientific Papers. 2016;45:209-229.
9. Vostroilova GA, Parshin PA, Khokhlova NA, Grigorieva NA. Study of the anti-inflammatory effect of tissue preparations in white mice. J Vet Pharmacol Ther. 2018;3:40-45.
10. Voronkov AV, Luzhnova SA, Kodonidi IP. Comparative analysis of the anti-inflammatory and analgesic effects of the acyclic 1,3-diazinone-4 precursor compound PNTd and the drug dapsone. Bulletin of Vol GMU. 2020;74:109-113.
11. Chernukh AM. Inflammation essays on pathology and experimental therapy. Medicine. 1979: pp:272-284.
12. Somova LM, Andryukov BG, Drobot EI, Lyapun IN. Granulomatous inflammation as a factor contributing to the persistence of the pathogen in *Yersinia pseudotuberculosis* infection. Clinical and Experimental Morphology. 2020;9:5-10.
13. Herzinger T, Berneburg M, Ghoreschi K. S1-guidelines on UV-phototherapy and photochemotherapy. J Dtsch Dermatol Ges. 2016;14:853-876.
14. Vengerovsky AI, Burkova VN, Yudina NV, Yatsenkova AI. Anti-inflammatory and analgesic effect of polar lipids of maral and peat antlers in experimental inflammation. Byull Siberia Medicine. 2012;6.
15. Baranova UA. Local puva therapy for psoriatic onychodystrophies. Lingvo-Science Magazine. 2018;12:6-8.
16. Rusak YE. Shortwave ultraviolet rays in the treatment of acne. Bulletin of SURSU Medicine. 2017;32:68-70.
17. Kiryanova VV, Egorova YS, Petrova EV. Psoriasis: The importance of physiotherapeutic factors in the complex therapy of chronic dermatosis. Bulletin of Avicenny. 2019;21:1.
18. Viola G, Salvador A, Vedaldi D, Dall'Acqua F, Bianchi N, Zuccato C, et al. Differentiation and apoptosis in UVA-irradiated cells treated with furocoumarin derivatives. Ann NY Acad Sci. 2009; 1171: 334-44.
19. Kruglova LS. Guidelines for the use of PUVA therapy in patients with chronic dermatoses. Moscow. 2012:26.
20. Fingar V. Vascular effect of photodynamic therapy. J Clin Laser Med Surg. 2009;14:5.
21. Bobrik YV. Peloidotherapy in complex rehabilitation treatment of patients with periodontal pathology. Physiotherapy and Balneology Bulletin. 2019;4:67-70.
22. Chen CH, Hwang TL. Isoflavones and anti-inflammatory constituents from the fruits of *Psorolea corylifolia*. Phytochemistry, 2017.
23. Kwon YJ, Shin S, Chun YJ. Biological roles of cytochrome 450 1A1,1A2 and 1B1 enzymes. Arch Pharm Res. 2021;44:63-83.
24. Song L, Yu B, Yang Z. The mechanism of psoralen and isopsoralen hepatotoxicity as revealed by hepatic gene expression profiling in SD rats. Basic Clin Pharmacol Toxicol. 2019;125:527-535.
25. Halestrap AP. What is the mitochondrial permeability transition pore. Journal Mol Cell Cardiol. 2009;46:821-831.

26. Brookes PS, YoonY, Robotham JL. Calcium, ATP and ROS: A mitochondrial love-hate triangle. Am Journal Physiol Cell Phsiol. 2004;287:817-833.

27. Sivitz WI, Yorek MA. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. Antioxid Redox Signal. 2010;12:537-577.

28. Abidova AD, Pazylov MK, Tseomashko NE, Iriskulov BU. Study of the effect of *Psoralea drupacea* bunge extract on calcium dependent channels of mitochondria. Biol Sci. 2020;18.