




<http://dx.doi.org/10.35630/2199-885X/2021/11/4.15>

# THERAPEUTIC EFFECT OF SINGLET OXYGEN ADMINISTRATION ON CRYSTALLIZATION OF RATS' BLOOD SERUM AT THERMAL TRAUMA

Received 26 July 2021;  
Received in revised form 25 August 2021;  
Accepted 27 August 2021

Andrew Martusevich<sup>1,2✉</sup> , Agata Perunova<sup>1</sup>,  
Constantin Karuzin<sup>3</sup> , Ivan Bocharin<sup>1,2</sup> ,  
Alexandra Nikolaeva<sup>1,2</sup>

<sup>1</sup> Privolzhsky Research Medical University, Nizhny Novgorod, Russia

<sup>2</sup> Nizhny Novgorod State Agricultural Academy, Nizhny Novgorod, Russia

<sup>3</sup> Bioniq Health-Tech Solutions Ltd., London, United Kingdom

✉ [cryst-mart@yandex.ru](mailto:cryst-mart@yandex.ru)

**ABSTRACT** — Biological and therapeutic effects of singlet oxygen have not been investigated yet. The aim of this paper is to estimate the influence of a short course (10 days) of singlet oxygen inhalations on crystallogenic properties of rats' blood serum. The experiment was performed on 30 male Wistar rats, randomly divided into three equal groups. The first group was intact. The animals of the 2<sup>nd</sup> (control) and 3<sup>rd</sup> (test) groups under combined anesthesia were subjected to thermal trauma. Starting from the day following the injury, the rats of the test group inhaled daily the air flow from a singlet oxygen generator during 10 days. Blood samples were obtained from the rats of all groups. Dried samples were evaluated visually for crystallizability, structure index, facia destruction degree, and marginal zone clarity, using respective scales. It is stated that singlet oxygen inhalations facilitate the elimination of negative transformations in blood crystallization induced by thermal trauma. It indicates the positive rehabilitation potential.

**KEYWORDS** — singlet oxygen, inhalations, crystallization, blood serum.

## INTRODUCTION

It is known that inhalations of a gas stream of singlet oxygen are perceived by a number of researchers as a means of rehabilitation that improves adaptive capabilities of the body in sports medicine and in the treatment of a number of therapeutic diseases [1, 2]. At the same time, the systemic and molecular mechanisms of its action are still poorly studied [2, 3]. In this regard, we conduct systematic studies aimed at verifying and deciphering the effects and mechanisms of the influence of singlet oxygen on biological systems in physiological and pathological conditions. Thus, it was shown on human blood samples that the treatment with singlet oxygen stimulates antioxidant

systems, the energy metabolism of red blood cells and optimizes the parameters of acid-base equilibrium [1, 4]. In healthy animals, a ten-day course of singlet oxygen inhalations also caused an antioxidant effect and activation of energy metabolism in the blood and tissues [5].

### *The aim of the study*

was to study the dynamics of the crystallogenic properties of rat blood serum during a course of singlet oxygen inhalation in the post-burn period.

## MATERIAL AND METHODS

The experiment was performed on 30 male Wistar rats (body weight – 200–250 g, 2–2,5 month old), randomly divided into three equal groups. The first group (n=10) was intact, the animals did not undergo any manipulations, but only once received blood from the hyoid vein. The animals of the 2<sup>nd</sup> (control) and 3 (main) groups under combined anesthesia ("Zoletil" + "Xylaver") were subjected to thermal trauma according to our method, standard local treatment was carried out [6]. Starting from the day following the injury, the rats of the main group were inhaled daily for 10 days with an air flow coming from a singlet oxygen generator. To create a gas mixture including singlet oxygen, the Airnergy Professional Plus device (Germany) was used [3]. The duration of each procedure was 10 min. The generator power is 100%. The next day after the completion of the full course of inhalations, blood samples were obtained from the sublingual vein in rats of this group for research. At the same time, blood was taken from animals of group 2. All blood samples were centrifuged at 1500 rpm for 15 min. Then, 100 mcl. of blood serum was applied to a slide and were prepared with the crystalloscopy method [6, 8]. The dried slides (facias) were evaluated morphologically and visuametrically [6]. The main indicators evaluated on a point scale were crystallizability (the density of crystalline elements), the structure index (characterizes the complexity of structure), the degree of destruction of the facias (indicator of the correctness of structures formation) and the severity of the marginal zone of the facia.

Experiments with animals were provided in accordance with the rules of the European Convention ET/S 129, 1986 and Directives 86/609 EEC.

Statistical processing of the results was performed using Statistica 6.1 program.

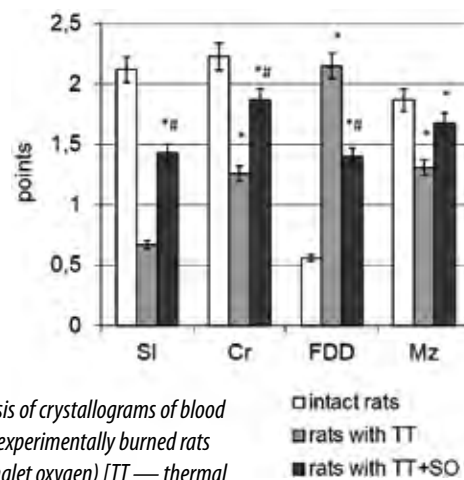
## RESULTS

A comparison of the crystalloscopic images of the blood serum of rats of the intact and control groups allowed us to confirm the previously shown patterns of transformation of the dehydration structuring of the biofluid associated with thermal trauma [6]. They are manifested in a pronounced inhibition of the crystallogenic activity of the biological medium, a simplification of the composition of elements and a sharp increase in the number and degree of destruction of the destroyed elements, as evidenced by shifts in the levels of crystallizability, the structural index and the degree of destruction of the facies, respectively. At the same time, the size of the marginal zone of the micropreparation in burned animals was significantly reduced, which is realized in a change in the corresponding morphometric indicator.

These trends of changes are realized in shifts of crystallogenic activity of animal blood serum (Fig. 1). Thus, a pronounced inhibition of the crystallization of biological fluid is indicated by a sharp drop in the level of crystallizability and the structural index in rats with thermal trauma compared with intact animals ( $p < 0.05$  for both indicators). Against this background, the processes of structure construction in drying samples of the biological environment are disrupted, which is clearly evidenced by a significant increase in the degree of destruction of the crystallogenic facies ( $p < 0.05$  in relation to healthy rats) — the main criterion of *correctness* [6]. Hypoproteinemia and dysproteinemia formed in burned animals are reflected in a decrease in the relative width of the marginal zone ( $p < 0.05$ ) containing proteins of the native structure and estimated by the level of the short-circuit parameter [9, 10, 13–15]. Conducting a course of inhalation therapy significantly reduces the severity of pathological shifts in the crystallogenic properties of rat blood serum (Fig. 1). It should be emphasized that the studied effect contributed to the normalization of all the indicators of the crystalloscopic test, and they statistically significantly differed both from the level characteristic of rats with thermal trauma and from the level of healthy animals ( $p < 0.05$  for all cases).

## CONCLUSION

In general, it was found that the inhalation of singlet oxygen in animals with severe thermal trauma contributes to the partial normalization of the crys-



**Fig. 1.** Criterion analysis of crystallograms of blood plasma in intact and experimentally burned rats (with and without singlet oxygen) [TT — thermal trauma, SO — singlet oxygen; SI — structure index, Cr — crystallizability, FDD — facia destruction degree, Mz — clarity of marginal zone; “\*” —  $p < 0.05$  to intact rats, “#” —  $p < 0.05$  to burned rats]

tallogenic activity of blood serum, which positively characterizes its rehabilitation potential.

## REFERENCES

- ZHANG D., GONG C., WANG J. ET AL. Unravelling Melatonin's Varied Antioxidizing Protection of Membrane Lipids Determined by its Spatial Distribution // *J Phys Chem Lett.* – 2021. – Vol. 12, N 31. – P. 7387–7393. doi: 10.1021/acs.jpcclett.1c01965.
- YANG D.C., WANG S., WENG X.L. ET AL. Singlet Oxygen-Responsive Polymeric Nanomedicine for Light-Controlled Drug Release and Image-Guided Photodynamic-Chemo Combination Therapy // *ACS Appl Mater Interfaces.* – 2021. – Vol. 13, N 29. – P. 33905–33914. doi: 10.1021/acsami.1c09044.
- HUANG X., CHEN T., MU N. ET AL. Supramolecular micelles as multifunctional theranostic agents for synergistic photodynamic therapy and hypoxia-activated chemotherapy // *Acta Biomater.* – 2021. – Vol. S1742–7061. P. 00445-1. doi: 10.1016/j.actbio.2021.07.014
- MONSOUR C.G., DECOSTO C.M., TAFOLLA-AGUIRRE B.J. ET AL. Singlet Oxygen Generation, Quenching, and Reactivity with Metal Thiolates // *Photochem Photobiol.* – 2021. doi: 10.1111/php.13487.
- MARTUSEVICH A.A., PERETYAGIN S.P., MARTUSEVICH A.K. Molecular and cellular effects of singlet oxygen on Biosystems // *Sovremennyye tehnologii v meditsine.* – 2012. – №2. – P. 128–134.
- VOROBYOV A.V., MARTUSEVICH A.K., SOLOVYOVA A.G. ET AL. Physical and biochemical characteristics of biological fluids in rats with modeled thermal injury // *Bulletin of Experimental Biology and Medicine.* – 2009. – Vol. 147, №4. – P. 424–426.