The Influence of Singlet Oxygen Inhalations on Rats with Experimental Burn Trauma

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Abstract: - This article aims to study the dynamics of the crystallogenic properties of rat blood serum during a course of singlet oxygen inhalation in the post-burn period. The experiment was performed on 30 mature Wistar rats randomly divided into three groups of equal numbers. The first group (n=10) was intact, no manipulations were performed on the animals included in it, but only a single blood irradiation from the sublingual vein was performed. The animals of the second (control) and third (main) groups under combined anesthesia were subjected to thermal trauma according to our methodology, standard local treatment was carried out. Starting from the day following the injury, the rats of the main group were inhaled daily for 10 days of the airflow coming from the singlet oxygen generator. Blood samples from rats of all groups were collected and tested for crystallogenic properties and crystallogenesis-inducing activity. It was found that the inhalation of singlet oxygen in animals with severe thermal trauma contributes to the partial normalization of the crystallogenic activity of blood serum, which positively characterizes its rehabilitation potential. This trend must take place when assessing both the intrinsic crystallogenic and initiating properties of biological fluid, which is manifested both in the dynamics of morphological and morphometric indicators and the optical characteristics of serum facies.

Key-Words: - singlet oxygen, inhalations, crystallization, blood serum.

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1 Introduction

It is known that inhalations of a gas stream initially containing singlet oxygen are perceived by several domestic and foreign researchers as a means of rehabilitation, contributing to an increase in the adaptive capabilities of the body in sports medicine and the treatment of several therapeutic diseases, [1], [2], [3]. At the same time, the systemic and molecular-cellular mechanisms of its action are still poorly understood, [2], [4]. In this regard, we are

conducting 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, human blood samples have shown that its treatment with singlet acid promotes the stimulation of antioxidant systems, energy metabolism of erythrocytes, and optimization of acid-base equilibrium parameters, [4]. In healthy animals, a ten-day course of singlet oxygen inhalation also caused an antioxidant effect and activation of energy metabolism in blood and tissues, [5]. The effect of the considered effect on the crystallogenic properties of blood serum realized in the form of structuring both in vitro and in vivo (on Wistar rats), has also been demonstrated, [6].

When modeling severe systemic pathology (on the example of thermal trauma) positive metabolic effects of singlet oxygen have also been established, while the effect on the dehydration structuring of blood serum has not been studied.

In this regard, the study aimed to study the dynamics of the crystallogenic properties of rat blood serum during a course of singlet oxygen inhalation in the post-burn period.

2 Materials and Methods

The experiment was performed on 30 mature Wistar rats randomly divided into three groups of equal numbers. Working conditions with rats corresponded to the rules of the European Convention ET/S 129, 1986, and Directives 86/609 ESC. The first group (n=10) was intact, no manipulations were performed on the animals included in it, but only a single blood irradiation from the sublingual vein was performed.

The animals of the second (control) and third (main) groups under combined anesthesia ("Zoletil" + "Xyla vet") were subjected to thermal trauma according to our methodology, standard local treatment was carried out, [7]. Starting from the day following the injury, the rats of the main group were inhaled daily for 10 days of the airflow coming from the 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 minutes. The power of the generator is 100%. The next day after the completion of the full course of inhalations in rats of this group, blood samples were obtained from the sublingual vein for examination. At the same time, blood was taken from animals of the second group.

To obtain blood serum, all samples were centrifuged at 1500 rpm for 15 minutes. Then the blood serum in the volume of 100 ml was applied to a slide and micro-preparations of dried biological fluid were prepared in accordance with the methods of crystalloscopy and comparative teziography, [6], [8]. 0.9% sodium chloride solution was used as the base substance in the tezigraphic test.

The dried micro-preparations were evaluated morphologically (by describing the features of the structuring of the dried sample of biological fluid), [8], [9], [10], [11], and visuametrically (using their system of parameters), [6]. The main visuometric indicators evaluated on a point scale were crystallizability (reflects the quantitative side of crystallization – the density of crystalline elements in the facies), the structure index (characterizes the complexity of structure construction), the facia destruction degree (is an indicator of the qualitative side of the process – the correctness of the formation of structures) and the clarity of the marginal zone of the micropreparation. For the criterion description of tezigrams, the maim teziographic index was used (it reflects the initiator potential in relation to the base substance), crystallinity (similar to the structure index), the facia destruction degree, and the clarity of the marginal zone.

The results of morphological and visuametric evaluation of blood serum micro-preparations were additionally verified using a spectroscopic examination of facies. The optical properties of the formed crystalloscopic and teziographic samples were analyzed using a “PowerWave XS” flatbed spectrophotometer (USA) at wavelengths of 300, 350, and 400 nm. Correction of the level of optical density of micropreparations was carried out by subtracting the empty glass from the obtained optical density value (registration was additionally performed on a section of each object glass that did not contain dehydrated drops of biological fluid).
Statistical processing of the results was performed using the licensed program Statistica 6.1 for Windows. The level of statistical significance of the differences was determined by the Student's t-criterion.

3 Results and Discussion

Comparison of crystalloscopic blood serum patterns of rats of the in-tact and control groups allowed us to confirm the previously shown patterns of transformation of dehydration structuring of biofluid associated with thermal trauma, [12].

They manifest themselves in a pronounced suppression of the crystallogenic activity of the biological environment, 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 index of structure and the degree of destruction of 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.

The indicated trends of changes in the nature of crystallogenic activity of animal blood serum are fully consistent with the results of parametric assessment of these facies (Figure 1). Thus, a pronounced inhibition of the crystallization of biological fluid is evidenced by a sharp drop in the level of crystallizability and structural index in rats with thermal trauma compared with intact animals ($p<0.05$ for both indicators). Against this background, the processes of structure-building in drying samples of the biological environment are disrupted, as evidenced by a significant increase in the degree of destruction of the crystallogenesis facies ($p<0.05$ in relation to healthy rats) – the main criterion of "correctness", [6]. Hypoproteinemia and dysproteinemia formed in burnt 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 clarity of marginal zone, [9], [10], [13], [14], [15].

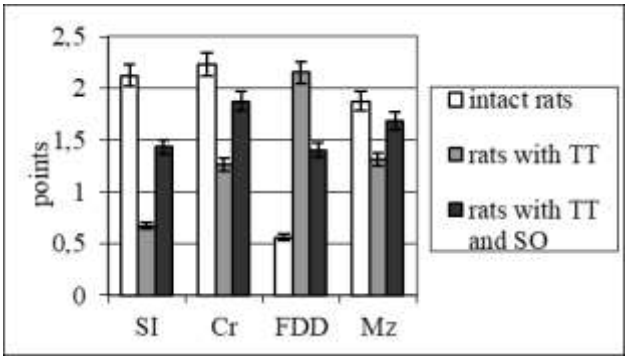

Fig. 1: Results of evaluation of blood serum crystallograms of rats of the formed groups (TT – thermal trauma, SO – inhalation of singlet oxygen, SI – structure index, Cr – crystallizability, FDD – facia destruction degree, Mz – clarity of the marginal zone)

Conducting a course of inhalation therapy significantly reduces the severity of pathological shifts in the crystallogenic properties of rat blood serum (Figure 1). It should be emphasized that the studied effect contributed to the normalization of all the main evaluation indicators of the crystalloscopic test, and they statistically significantly differed from both the level characteristic of rats with thermal trauma and from the level of healthy animals ($p<0.05$ for all cases). This indirectly indicates the adaptive capabilities of the tested method of metabolic correction of disorders occurring in the post-post period.

Similar nature of changes was recorded in the analysis of the results of co-crystallization of the blood serum of animals with a basic substance evaluated in the teziographic test (Figure 2). In this case, the rats that received thermal trauma also observed a pronounced inhibition of the initiatory properties of the biological environment compared with intact animals, as evidenced by a significant decrease in the level of the tezigraphic index and crystallinity in facies ($p<0.05$ for both indicators). In addition, there was an increase in the degree of destruction of the sample elements in combination with a decrease in the size of the marginal zone of the micropreparation ($p<0.05$ relative to healthy rats).

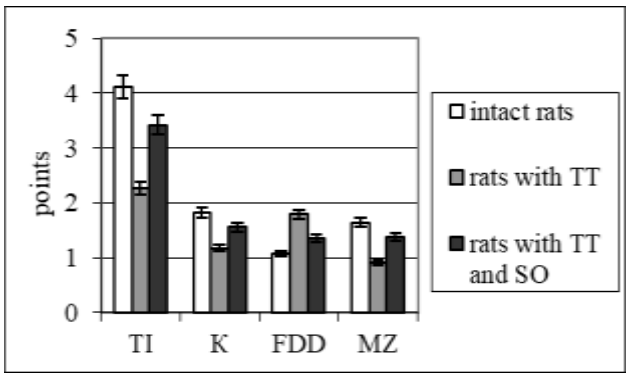

Fig. 2: The results of the evaluation of blood serum tezigrams of rats of the formed groups (The base substance is 0.9% sodium chloride solution; TI – teziographic index, K - crystallinity; the remaining abbreviations are as in Figure 1)

The criterion evaluation of blood serum tezigrams of the animals of the main group who received a course of singlet oxygen inhalation after thermal injury was completely consistent with the results of the crystalloscopic study because, for all the main indicators, a statistically significant approximation to the values characteristic of the intact group was noted. The most pronounced trend was in relation to the crystallinity of the samples and the severity of the marginal zone, which may indirectly indicate a decrease in the severity of endogenous intoxication present in animals, as well as a significant normalization of the protein profile

of the blood. It should be emphasized that the second of the above parameters after a course of singlet oxygen injections was found to be only slightly reduced in comparison with healthy animals ($p<0.1$). At the same time, the high severity of dysmetabolic shifts induced by combined thermal trauma inflicted on rats contributed to the preservation of a significantly reduced level of the tezigraphic index, as well as a moderate tendency to optimize the degree of destruction of facies after a course of treatment supplemented by inhalation use of a singlet-oxygen gas mixture.

In general, the data of morphological and visuometric evaluation of crystallograms and tezigrams of rat blood serum indicate a positive effect of singlet oxygen inhalations on these physico-chemical parameters.

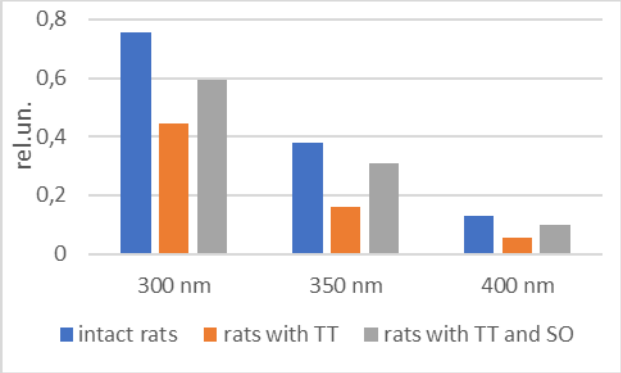

Fig. 3: The level of optical density of blood serum crystallograms of rats of the formed group's groups (TT – thermal trauma, SO – inhalation of singlet oxygen)

To verify the results of basic crystalloscopic studies, we applied spectrometric analysis of crystallographic and tezigraphic facies of animal blood serum (Figure 3 and Figure 4).

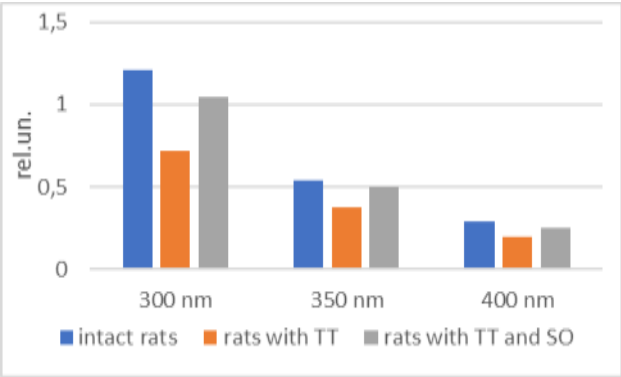

Fig. 4: The level of optical density of blood serum tezigrams of rats of the formed group's groups (TT – thermal trauma, SO – inhalation of singlet oxygen)

It was found that in rats with thermal trauma, the optical density of crystallograms is significantly lower than in healthy animals ($p<0.05$), and this trend occurs at all wavelengths used and is expressed approximately equally with them (Figure 3). This observation is consistent with the results of basic crystallographic studies, which show a significant decrease in the number of structural elements, as well as complexity and construction, which is realized in the form of a statistically significant decrease in crystallizability and the structural index, respectively ($p<0.05$).

Inhalation of the singlet oxygen airflow contributes to partial restoration of the optical density of micro-preparations of dehydrated rat blood serum, which also occurs at all studied wavelengths, which confirms the results of the crystalloscopic study.

Similar shifts in optical density were registered concerning blood serum tesigrams of rats of the control and main groups (Figure 4). At the same time, they were less pronounced compared with those characteristic of biological fluid crystallograms.

4 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 crystallogenic activity of blood serum, which positively characterizes its rehabilitation potential. This trend must take place when assessing both the intrinsic crystallogenic and initiating properties of biological fluid, which is manifested both in the dynamics of morphological and morphometric indicators and the optical characteristics of serum facies.

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- Andrew K. Martusevich: Conceptualization, Investigation, Formal analysis, and Writing – original draft and Writing – review & editing.
- Roman A. Kornev: Conceptualization, Formal analysis, and Writing – original draft.
- Lida K. Kovaleva; Elena A. Stepanova: Investigation, Formal analysis
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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.