ASSESSMENT OF THE ENERGY STATUS OF THE BLOOD OF BURN PATIENTS UNDER THE INFLUENCE OF HYPEROXIA AND ANTIOXIDANTS

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Abstract. The energy shortage, as well as the lack of antioxidant micronutrients that occur with severe thermal lesions, lead to poor clinical outcomes. It is important to carry out timely and effective therapy that helps maintain the redox and energy balance of the burned body. The aim of this work was to evaluate the energy status of red blood cells in severely burned patients with the use of antioxidant therapy and hyperbaric oxygenation on different days of therapy. The study involved 60 practically healthy people (norm) and patients with burns of I-II-III degree with different localization over 25% of the body surface. The age of patients is from 20 to 87 years. The data obtained suggested the effectiveness of the use of antioxidant therapy in burn patients due to the activation of oxidoreductases that support energy metabolism. The combined use of HBO and antioxidant nutrients in addition to standard therapy presumably contributed to maintaining the energy and redox balance of the body in patients with burns.

Keywords: burn, hyperbaric oxygenation, energy metabolism, antioxidants.

List of Abbreviations

HBO – hyperbaric oxygenation

GR – glutathione reductase

Gl-6-phDH – glucose-6-phosphate dehydrogenase

LDH direct – lactate dehydrogenase in direct reaction

LDH reverse – lactate dehydrogenase in reverse reaction

OS – oxidative stress

Introduction

One of the most difficult problems in combustiology is the treatment of victims with extensive burns (Glutkin & Koval'chuk, 2016). Extensive skin damage and serious systemic complications (hypoxia, systemic inflammatory reaction syndrome, multiple organ failure, sepsis) occur with severe thermal lesions. They are the leading causes of morbidity and mortality in this pathology (Moreira *et al.*, 2018, Prauchner, 2017). It is believed that the pathogenesis of post-burn injury arises from an abnormal accumulation of lipids due to the development of a persistent hypermetabolic and hypercatabolic state. As a result, there is an accumulation of reactive oxygen species and the development of OS (Dkhil *et al.*, 2015, Dryden, 2017).

Calorie deficiency, negative protein balance and deficiency of antioxidant micronutrients after thermal injury are associated with poor clinical outcomes. Therefore, it is important to carry out timely and effective therapy that helps maintain the redox and energy balance of the burned body (Beiraghi-Toosi *et al.*, 2018).

The main strategies for treating burns include measures to minimize edema, maintain the viability of tissues in the stasis zone of the burn, protect the microcirculatory bed and strengthen the body's defenses to prevent infection (Babu & Babu, 2018). HBO reduces the manifestation of these physiological disorders in burn patients, weakens inflammatory and oxidative reactions, reduces oxygen starvation of tissues (Alyafi *et al.*, 2021, De Wolde *et al.*, 2021). The effectiveness of HBO therapy in improving neovascularization and ischemia, reperfusion injury and reducing swelling of burn wounds is known (Lindenmann *et al.*, 2021, Olifirova & Kozka, 2017). Thus, this therapy can be used as part of a treatment regimen to reduce healing time and improve results (Levina *et al*., 2020).

It is also important to note the depletion of components of the antioxidant defense system of the body of patients with burns, due to the development of OS (Ighodaro & Akinloye, 2017). The additional use of antioxidant micronutrients in burns can have a protective effect in oxidative tissue damage. Moreover, the earlier the treatment is prescribed, the more effective such therapy is in severely burned patients (Kolesnikov *et al*., 2020).

The aim of this work was to evaluate the energy status of red blood cells of patients with burns under the influence of antioxidant therapy and hyperbaric oxygenation.

Materials and Methods

The study involved 60 practically healthy people (norm) and 25 patients with burns of I-II-III degree over 25% of the body surface. The age of patients is from 20 to 87 years. The study was conducted in accordance with the standards of good clinical practice and the principles of the Helsinki Declaration of 1975 and its revised version of 2000. Prior to inclusion in the study, written informed consent was obtained from all participants, and the study itself was approved by the Local Ethics Committee. The first group consisted of 10 patients with standard methods of treatment adopted in the clinic of thermal lesions. The second group consisted of 10 patients with standard therapy and a course of antioxidant therapy (parenteral administration of Cernevit and Addamel H). Group 3 included 5 patients with combination therapy (standard therapy + HBO + antioxidant therapy). Hyperbaric oxygenation was performed in pressure chambers BLKS-30, BLKS-307/1 with an extended isopression regime of 1.3 Ata with a total session duration of 50–60 minutes

The activity of GR, Gl-6-phDH, LDH was determined in erythrocyte hemolysate (1:40). The method for determining the activity of GR is based on a change in the absorption of the solution during the formation of the oxidized form of NADP +. The activity of Gl-6-phDH was determined using spectrophotometric analysis. The principle of the method is based on determining the amount of NADPH formed during the oxidation of glucose-6-phosphate into phosphoglucolactone. LDH activity in direct and reverse reactions was determined by the spectrophotometric method based on the assessment of the oxidation rate of NADH. To calculate the specific activity of these oxidoreductases, the protein concentration in each sample was determined by the spectrophotometric method (Soloveva, 2020).

Statistical data processing was performed using software (Statistica 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA)). The normality of the distribution of results was shown using the Shapiro-Wilk criterion. The significance of the differences between the groups was assessed using the Student's t-test and one-sided analysis of variance (ANOVA). The differences were considered statistically significant at $p < 0.05$.

Results

Since tissue damage caused by a burn is acute or prolonged, it is possible to indirectly assess the level of damage by the activity of the enzyme of energy metabolism – LDH. As a result of the conducted studies, a statistically significant decrease in the specific activity of LDH direct in erythrocytes in group 1 patients was shown by an average of 26–49% on the 2nd, 4th and 10th days after the burn compared with the norm (Table 1). This indicated the likely persistence of hypoxia in the blood of these patients.

In the 2nd group of patients, a decrease in the LDH direct activity in red blood cells was found only on the 1st day after injury by 32.063% ($p = 0.011$) compared to the norm. A statistically significant increase in the specific activity of the enzyme on days 4 and 10 was shown by 23.75% and 28.15%, respectively, compared with the level of the indicator of group 1 patients.

For patients of group 3, there was a tendency to normalize the specific activity of LDH direct on almost all the studied days. At the same time, the level of the indicator was higher by 24.70% ($p = 0.015$) and 30.53% ($p = 0.021$) on the 2nd and 4th days after the burn, respectively, compared with patients of group 1. There was also a statistically significant increase in the specific

Table 1

Evaluation of the dynamics of specific activity of lactate dehydrogenase in direct reaction in erythrocytes depending on the time after injury and type of treatment

τ, Day	LDH direct activity, % of norm[1]		
	Group 1	Group 2	Group 3
1	66.908 ± 2.658	67.937 ± 3.011*	77.658 ± 3.745*
2	74.193 ± 3.254*	75.543 ± 3.592	98.891 ± 4.298**/***
3	71.102 ± 3.566	82.414 ± 4.029	89.016 ± 3.826
4	61.244 ± 2.933*	84.998 ± 3.955**	91.777 ± 4.011**
7	77.973 ± 3.621	86.465 ± 3.716	86.889 ± 3.762
10	51.560 ± 2.292*	79.714 ± 3.861**	73.302 ± 3.135*/**
14	75.363 ± 3.359	72.791 ± 3.714	98.786 ± 4.139***

Note: [1] 100% - 54.18 ± 5.091 nmol NADH/min*mg protein;
* – the values are statistically significant compared to the norm ($p < 0.05$);
** – the values are statistically significant compared to group 1 ($p < 0.05$);
*** – the values are statistically significant compared to group 2 ($p < 0.05$).

Table 2

Evaluation of the dynamics of the specific activity of lactate dehydrogenase in reverse reaction in erythrocytes depending on the time after injury and type of treatment

τ, Day	LDH reverse activity, % of norm[1]		
	Group 1	Group 2	Group 3
1	92.705 ± 3.026	86.195 ± 3.144**	72.605 ± 2.001*
2	89.592 ± 2.945*	88.992 ± 3.265	88.039 ± 2.822*
3	93.626 ± 3.159	88.877 ± 3.254	94.336 ± 3.343
4	93.168 ± 2.867	94.482 ± 3.677	94.277 ± 3.567
7	97.297 ± 3.648	90.342 ± 3.545	83.036 ± 2.956
10	89.67 ± 2.455*	92.958 ± 3.069	96.741 ± 3.213
14	95.427 ± 3.987	96.423 ± 3.871	96.423 ± 3.562

Note: [1] 100% - 303.45 ± 12.205 nmol NADH/min*mg protein;
* – the values are statistically significant compared to the norm ($p < 0.05$);
** – the values are statistically significant compared to group 1 ($p < 0.05$);
*** – the values are statistically significant compared to group 2 ($p < 0.05$).

activity of the enzyme on days 2 and 14 by an average of 23–26% compared with the level of the indicator in patients of group 2.

Thermal trauma contributed to a slight decrease in the specific activity of LDH and in the reverse reaction on all the studied days (Table 2).

However, it is worth noting the normalization of the specific activity of lactate dehydrogenase in the reverse reaction in all 3 groups of patients, which indicated the effectiveness of the treatment and the prevention of hypoxia and energy imbalance.

The burn injury contributed to the increase in the activity of other enzymes of energy metabolism. Thus, there was an increase in the specific activity of Gl-6-phDH, a key enzyme of the pentose phosphate pathway (Table 3).

There was a statistically significant increase in the specific activity of Gl-6-phDH in the

Table 3

Evaluation of the dynamics of the specific activity of glucose-6-phosphate dehydrogenase in erythrocytes depending on the time after injury and type of treatment

τ, Day	Gl-6-phDH activity, % of norm[1]		
	Group 1	Group 2	Group 3
1	103.402 ± 5.216	100.981 ± 5.312	95.312 ± 4.358
2	113.376 ± 6.333	104.979 ± 5.749	95.999 ± 4.637
3	103.47 ± 5.144	105.851 ± 5.984	110.982 ± 6.023
4	91.522 ± 4.317	100.848 ± 4.912	93.166 ± 5.264
7	104.116 ± 5.647	105.796 ± 4.587	99.948 ± 5.269
10	111.373 ± 6.216*	100.851 ± 5.014	131.985 ± 6.111*/**/***
14	115.915 ± 6.874*	100.055 ± 4.876	110.192 ± 6.035*

Note: [1] 100% – 52.384 ± 3.571 nmol NADPH/min*mg protein;
* – the values are statistically significant compared to the norm ($p < 0.05$);
** – the values are statistically significant compared to group 1 ($p < 0.05$);
*** – the values are statistically significant compared to group 2 ($p < 0.05$).

Table 4

Evaluation of the dynamics of specific glutathione reductase activity in erythrocytes depending on the time after injury and type of treatment

τ, Day	GR activity, % of norm[1]		
	Group 1	Group 2	Group 3
1	100.341 ± 5.299	87.388 ± 5.234	89.279 ± 7.246
2	104.411 ± 3.112	87.947 ± 4.987	103.829 ± 5.321
3	84.319 ± 4.211*	91.191 ± 5.762	102.040 ± 8.265**
4	84.319 ± 8.012*	90.615 ± 3.954	108.391 ± 6.347**/***
7	97.569 ± 8.002	94.451 ± 5.891	106.909 ± 5.278**/***
10	79.476 ± 6.277*	82.232 ± 4.021*/**	109.596 ± 6.548*/***
14	94.388 ± 7.414	80.391 ± 4.388*	93.549 ± 4.987

Note: [1] 100% – 79.273 ± 3.856 nmol NADPH/min*mg protein;
* – the values are statistically significant compared to the norm ($p < 0.05$);
** – the values are statistically significant compared to group 1 ($p < 0.05$);
*** – the values are statistically significant compared to group 2 ($p < 0.05$).

blood erythrocytes of group 1 patients on days 10 and 14 by 11.37% and 15.92%, respectively, compared with the norm. The activity of Gl-6-phDH in group 2 patients was normal on all the studied days. And in patients of group 3, the specific activity of the Gl-6-phDH increased on days 10 and 14 by 31.99% ($p = 0.010$) and by 10,19% ($p = 0.017$), respectively, compared with the norm. Such an increase in enzyme activity may have contributed to the accumulation of a pool of NADPH necessary for the proper functioning of the antioxidant system, especially for its enzyme such as GR.

Table 4 shows the data of the study of the dynamics of the specific activity of GR in the erythrocytes of the blood of burned patients, depending on the time after injury and type of treatment.

It was noted that the specific activity of GR in the blood erythrocytes of group 1 patients was statistically significantly reduced by 3, 4 and 10 days on average by 16–21% compared to the norm. In patients of group 2, the specific activity of the enzyme was also reduced on days 10 and 14 by 17.77% ($p = 0.021$) and by 19,61% ($p = 0.013$), respectively, compared with the norm. GR is responsible for recycling glutathione, a free radical scavenger. These data are consistent with the idea that metabolic changes in the body after a burn are partially associated with a deficiency of antioxidant protection.

However, the GR activity in the blood erythrocytes of group 3 patients was statistically significantly higher on 3, 4 and 7 days under study by an average of 9–17% compared with the GR activity in group 1 patients. A similar trend was observed when comparing the specific activity of the enzyme on 4, 7 and 10 days after injury.

Thus, only in patients of group 3, the activity of oxidoreductases was increased, contributing to the reduction of oxidative stress and acceleration of metabolism in the burned body.

Discussion

In this paper, the possibility of using antioxidant therapy (Cernevit and Addamel) and HBO along with standard treatment methods adopted in the clinic of thermal lesions was studied. Damage to cells and tissues resulting from thermal trauma was indirectly assessed by changes in the activity level of the enzyme of energy metabolism, lactate dehydrogenase. LDH converts lactate to pyruvate in a direct reaction and pyruvate to lactate in a reverse reaction. In response, the process of oxidative phosphorylation switches to glycolysis, and energy production continues in a relatively hypoxic environment (Woo *et al*., 2020). Human red blood cells have significant LDH activity, and the enzyme itself is important in maintaining the ratio of oxidized and reduced forms of NAD in the cell (Thiele, 2017). The data obtained indicated an acceleration of the processes of energy metabolism in burn patients receiving additional treatment with antioxidant nutrients, which was manifested by an increase in LDH activity.

Severe burn injury is known to increase energy consumption, including due to the restriction of phosphorylation (Foncerrada *et al*., 2018). It was noted that treatment with antioxidant nutrients contributed to an increase in activity of Gl-6-phDH. This enzyme limits the speed of the pentose phosphate pathway, plays a key role in lipid metabolism, and is the main source of NADPH. NADPH is required by many important cellular systems that ensure cell survival, including antioxidant pathways, nitric oxide synthase, NADPH oxidase, cytochrome p-450 system, and others (Spencer & Stanton, 2017).

Standard methods of treatment of burn patients were less effective in maintaining the energy and redox balance of the burned body.

According to the literature data, HBO is a method included in the intensive treatment of burn disease and wounds. HBO increases oxygen delivery to tissues, reduces the development of wound infections, reduces the development of multiple organ dysfunction (Bosco *et al*., 2018, Cejka & Cejkova, 2015). The proposed method of therapy, including antioxidants and HBO, was pathogenetically conditioned and suggested a positive effect on the course of the reparative process in burn wounds, manifested in a significant induction of enzymes of energy metabolism (Edwards *et al*., 2022, Hatibie *et al*., 2019). An increase in wound healing, in turn, would contribute to a reduction in mortality, a reduction in rehabilitation time and a reduction in the need for surgical intervention (Weitgasser *et al*., 2021). Studies have revealed that HBO stimulates the intensity of tissue metabolism. Additional therapy with antioxidant nutrients contributed to ensuring the proper functioning of the antioxidant system by increasing the pool of NADPH, and activation of such an enzyme of the antioxidant system as GR.

Conclusions

The data obtained suggest the effectiveness of the use of antioxidant therapy in patients with burns due to the activation of oxidoreductases that support energy metabolism. The combined use of HBO and antioxidant nutrients presumably contributes to maintaining the energy and redox balance of the blood during thermal trauma.

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