

Some Aspects of the Mechanism of the Antihypoxant Action of Glisimed

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Abstract The experimental studies which were conducted on mature white male mice, showed that normobaric hypoxia was accompanied by significant changes in carbohydrate metabolism, in particular, there were an increase in the level of glucose, pyruvic and lactic acid. Glisimed, phytin and piracetam have a corrective effect on the parameters of carbohydrate metabolism in the posthypoxic period, but they differ in their pharmacological activity. Along with this, it was found that normobaric hypoxia is accompanied by significant changes in metabolism leading to the accumulation of toxic metabolic products in the body, which is expressed in the development of endogenous intoxication syndrome. The studied medicines eliminate the developed enotoxemia, which is expressed in a decrease in the sorption capacity of erythrocytes and the level of medium molecular peptides, as well as in the prolongation of the life span of paramecium. Some superiority of glisimed in comparison with other medicines has been revealed.

Keywords Hypoxia, Carbohydrate metabolism, Endotoxemia, Antihypoxants, Medicinal plants

1. Introduction

One of the universal pathological processes underlying and determining the development of the most diverse pathology is hypoxia [1,2,3,4]. This typical pathological process is a condition in which there is a discrepancy between the energy demand of the cell and energy production in the system of oxidative phosphorylation occurring in mitochondria. Despite the variety of causes leading to hypoxia, a change in the oxidation of energy substrates unequivocally develops: a transition to an anaerobic oxidation cycle [5]. The anaerobic oxidation process, as a rule, is accompanied by an abundance of accumulation of underoxidized products, which aggravates the course of the pathological process [6]. It is known that oxygen deficiency activates anaerobic glycolysis, which partially compensates for the lack of adenosine triphosphate, and maintains the normal functioning of cells for a limited time. However, in the vast majority of cases, the development of metabolic acidosis and hyperlactonemia is the end result of hypoxia regardless of its type. Therefore, lactate, being the end product of anaerobic metabolism, serves as a sensitive marker of hypoxia [4,7]. We have previously shown that the sum of extracts of medicinal plants - *Hipericum scabrum* L., *Ziziphora pedicellata* Pazij

Vved., *Mediaria macrophylla* has a pronounced antihypoxant effect [8]. However, the mechanism of the pharmacological activity of glisimed and, in particular, its influence on the characteristics of carbohydrate metabolism during hypoxia remained unexplored.

The purpose of this work was to study the effect of glisimed on carbohydrate metabolism and endogenous intoxication syndrome in normobaric hypoxia.

2. Material and Methods

Experimental studies were carried out on male white mice of herd breeding with a body weight of 19-23 g, which were obtained from the vivarium of the Department of Sanitary and Epidemiological Surveillance of the Main Medical Department under the Administration of the President of the Republic of Uzbekistan. After quarantine for 14 days, all laboratory animals were carefully examined, weighed, their age, sex, and motor activity were taken into account. The laboratory animals were kept in a vivarium at a temperature of 20-24°C, humidity of at least 50%, in a well-ventilated room and day/night light regimen, in standard plastic cages of 8-10 individuals in each, with a standard diet during the entire period of preparation for the experiment and during its implementation. The method of obtaining a dry extract from medicinal plants was carried out in the same way as described by us earlier [8].

2.1. Experiments

The model of normobaric hypoxia with hypercapnia

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was modeled by placing animals in a hermetic chamber with a volume of 250 cm³ [8,9,10]. The mixture of extracts of medicinal plants- *Hypericum scabrum* L., *Ziziphora pedicellata* Pazij Vved., *Mediizia macrophylla* (conditionally named glisimed) at doses of 10, 25 and 50 mg/kg, piracetam - 100 mg/kg and fitin - 200 mg/kg s were intragastrically administered to the animals of the experimental group one day and one hour before modeling of normobaric hypoxia with hypercapnia. When the first symptoms of forthcoming death appeared, the animals were removed from the hermetic chamber. After 1, 6, and 24 hours of the posthypoxic period, the animals were decapitated under light ether anesthesia and blood was collected for biochemical studies. The content of increased concentration of glucose in the blood was determined using a set of reagents from CYPRESS Diagnostics, Belgium) on a biochemical analyzer BA-88A (Mindray, China), pyruvic acid (PVA) and lactic acid (LA) were determined according to the conventional method [11]. The development and degree of endogenous intoxication was judged by the sorption capacity of erythrocytes, the level of medium molecular peptides and the paramecium test. The sorption capacity of erythrocytes (SCE) was determined by the method of A.A.Togaibaev *et al.* [12]. The principle of the method is based on the fact that under the influence of endogenous toxins, the ability of erythrocytes to absorb vital dyes, such as methylene blue, increases significantly. The optical density of the dye solution before and after the deposition of erythrocytes was determined on a spectrophotometer at a wavelength of 630 nm. Determination of the content of medium molecular peptides (SMPs) was determined by the method of N.I. Gabriellan *et al.* [13]. The proposed method consists in the precipitation of coarsely dispersed proteins by 10% trichloroacetic acid, followed by the detection of the eluting fraction at a spectrophotometer wavelength of 254 nm. The level of "medium molecules" in all series of studies was expressed in conventional units, quantitatively corresponding to the extinction data. Determination of the toxicity of blood serum according to the paramecium test was carried out by the method of V.S. Genes *et al.* [14]. In experiment, 0.01 ml of blood serum containing from 8 to 10 paramecium was mixed in the melangeur for erythrocytes. After the addition of blood serum, the time of death of half, then all paramecium was determined and the results were expressed in seconds.

Experimental studies were carried out in accordance with the "Rules for Conducting Work Using Experimental Animals", as well as the rules adopted by the European Convention for the Protection of Vertebrate Animals used for experimental studies or for other scientific purposes (ETS No. 123), Strasbourg, 18.03.1986.

2.2. Statistical Analysis

The data obtained were processed by the method of variation statistics using the paired Student's test and one-way analysis of variance using the standard software

package BIOSTAT 2009 with an assessment of the significance of indicators ($M \pm \text{Std. error}$). Differences in the compared groups were considered significant at a significance level of 95% $p < 0.05$.

3. Results and Discussion

It is known that energy supply of tissues and systems in extreme conditions, primarily provided by glucose, which is rightfully considered the primary substrate of energy metabolism [15]. As a rule, glucose oxidation occurs in the anaerobic phase in oxygen deficiency, which is accompanied by the formation of pyruvic acid (PVA) and lactic acid (LA) in the tissues. The metabolic acidosis developing at the same time aggravates the severity of the extreme condition. Since antipoxants eliminate these disorders of carbohydrate metabolism, favorable conditions are created for the functional activity of cells and subcellular structures, especially the brain tissue. Considering the above, the effect of glisimed on the content of glucose, LA and PVA in blood serum in animals subjected to normobaric hypoxia was studied for establishing the mechanism of the antihypoxic action of glisimed. The choice of blood as a biological object was due to the fact that inhibition of the aerobic phase of oxidation in the presence of oxygen deficiency leads to the release of PVA and LA from the tissue into the blood [4,5,6].

The results of the experimental studies showed that normobaric hypoxia is accompanied by significant changes in carbohydrate metabolism, in particular, after 1 hour of the posthypoxic period, there was an increase in the level of glucose by 65.0%, LA - 237.0% and PVA almost in 5.4 times compared with healthy individuals in experimental animals. As can be seen from the data in Table 1, these changes persist with slight fluctuations even after 6 hours from the onset of the impact of the extreme factor. However, carbohydrate metabolism disorders remain at a statistically significant level even after 1 day, where the concentration of LA and PVA increased by 2.16 and 2.35 times, respectively.

Consequently, normobaric hypoxia is accompanied by the disorders of carbohydrate metabolism, which retain at a significant high level even 24 hours after impact of the pathogenic factor.

The above changes indicate the need for the use of medicines for correction. Proceeding from this, we conducted studies, where the animals of experimental group received antioxidants preventively. It should be noted that the disturbances of carbohydrate metabolism in antioxidant received animals had the same direction as control animals, but their level was somewhat less.

Thus, under the influence of piracetam, the concentration of glucose increased by 26.6% after 1 hour from the beginning of the experiment, but in comparison with the control, it was low by 23.1%. It is noteworthy that the level of increasing of glucose in blood gradually weakened in the following hours. On this background, 1, 6 and 24 hours after exposure to extreme factor, the level of LA decreased by

32.4%, 39.6% and 30.2%, and PVA decreased respectively by 43.3%, 44.9% and 26.0% compared with the control animals. It can be seen that piracetam has a clear eliminating effect on the parameters of carbohydrate metabolism in the posthypoxic period. There were also distinct changes indicating the elimination of carbohydrate metabolism disorders in the group of animals receiving phytin preventively. Thus, after the stopping of hypoxia, the concentration of glucose in the indicated periods of observation decreased by 22.0%, 20.8% and 10.0%, respectively, LA - by 38.8%, 40.7% and 32.6%, and PVA - by 45.5%, 51.5% and 37.4% in comparison with untreated animals. It can be seen that phytin is to some extent superior by its activity then piracetam. We found a higher corrective effect in a new compound - glisimed. In animals treated with this drug, the increasing of the level of glucose concentration

in blood was only 17.2%, 11.0% and 5.9% compared with the control animals. At the same time, the value of the glucose level did not statistically significantly differ from healthy animals in the last two periods of observation,. It is noteworthy that on this background, under the influence of glisimed, the concentration of LA and PVA was less by 40.3% and 48.9% after 1 hour, by 41.8% and 55.7% after 6 hours, and also by 37.1% and 46. 3% after 24 hours, respectively. An analysis of the presented materials suggests that the studied medicines have a unidirectional effect on the parameters of carbohydrate metabolism in the post-hypoxic period, but they differ somewhat in their pharmacological activity. In this regard, the medicines can be put in descending order in the following row: glisimed > phytin > piracetam. The obtained results allow us to confirm some links of the mechanism of the antihypoxic action of glisimed.

Table 1. The effect of glisimed, phytin and piracetam on the content of glucose, lactic and pyruvic acid in blood serum during normobaric hypoxia in different hours of the study ($M \pm \text{Std.error}$, $n=6$)

indexes groups	hours of study	glucose, mg%	Lactic acid, mg%	pyruvic acid, mg%
Intact	-	5,93±0,27	2,56±0,23	0,084±0,007
Control	1	9,77±0,57 P <0,002	8,63±0,72 P <0,001	0,538±0,036 P <0,001
	6	8,88±0,46 P <0,01	8,01±0,52 P <0,001	0,499±0,041 P <0,001
	24	7,52±0,23 P <0,01	5,52±0,19 P <0,001	0,281±0,014 P <0,001
Glisimed	1	6,95±0,31 P <0,05 P ₁ <0,01	5,15±0,37 P <0,002 P ₁ <0,01	0,275±0,018 P <0,001 P ₁ <0,002
	6	6,58±0,29 P >0,05 P ₁ <0,01	4,66±0,29 P <0,01 P ₁ <0,01	0,221±0,016 P <0,001 P ₁ <0,002
	24	6,28±0,24 P >0,05 P ₁ <0,02	3,47±0,14 P <0,02 P ₁ <0,001	0,151±0,010 P <0,01 P ₁ <0,001
Pirasetam	1	7,51±0,42 P <0,05 P ₁ <0,05	5,83±0,29 P <0,001 P ₁ <0,02	0,305±0,026 P <0,001 P ₁ <0,01
	6	7,12±0,31 P <0,05 P ₁ <0,05	4,84±0,37 P <0,01 P ₁ <0,01	0,275±0,021 P <0,001 P ₁ <0,01
	24	6,93±0,28 P <0,05 P ₁ >0,05	3,85±0,28 P <0,02 P ₁ <0,01	0,208±0,018 P <0,002 P ₁ <0,05
Phytin	1	7,65±0,35 P <0,02 P ₁ <0,05	5,28±0,33 P <0,001 P ₁ <0,01	0,293±0,019 P <0,001 P ₁ <0,01
	6	7,03±0,19 P <0,05 P ₁ <0,02	4,75±0,25 P <0,002 P ₁ <0,01	0,242±0,015 P <0,001 P ₁ <0,002
	24	6,77±0,16 P <0,05 P ₁ <0,05	3,72±0,21 P <0,02 P ₁ <0,002	0,176±0,013 P <0,002 P ₁ <0,01

Note: P – statistically significant in comparison with intact,

P₁ - statistically significant in comparison with the control, corresponding hour of the study

Table 2. Influence of glisimed, phytin and piracetam on the syndrome of endogenous intoxication in normobaric hypoxia in different hours of study ($M \pm \text{Std.error}$, $n=6$)

indexes groups	Hours of study	sorption capacity erythrocytes, %	medium molecular peptides, CU	paramecium test, seconds
Intact	-	58,83 \pm 2,41	0,256 \pm 0,023	213,17 \pm 7,44
Control	1	87,67 \pm 5,57 P <0,01	0,545 \pm 0,035 P <0,001	130,83 \pm 4,71 P <0,001
	6	82,33 \pm 4,31 P <0,01	0,495 \pm 0,033 P <0,002	144,33 \pm 6,55 P <0,001
	24	78,83 \pm 3,27 P <0,01	0,455 \pm 0,021 P <0,002	160,33 \pm 3,27 P <0,002
Glisimed	1	64,17 \pm 1,70 P >0,05 <0,01	0,465 \pm 0,029 P <0,01 >0,05	158,66 \pm 5,04 P <0,002 <0,01
	6	61,67 \pm 1,53 P >0,05 P ₁ <0,01	0,393 \pm 0,028 P <0,02 P ₁ <0,05	174,67 \pm 3,42 P <0,01 P ₁ <0,01
	24	60,17 \pm 1,55 P >0,05 P ₁ <0,01	0,305 \pm 0,018 P >0,05 P ₁ <0,01	183,66 \pm 4,93 P <0,05 P ₁ <0,02
Piracetam	1	69,16 \pm 3,40 P <0,05 P ₁ <0,05	0,512 \pm 0,037 P <0,002 P ₁ >0,01	140,50 \pm 2,43 P <0,001 P ₁ >0,01
	6	66,50 \pm 2,48 P >0,05 P ₁ <0,05	0,478 \pm 0,014 P <0,001 P ₁ >0,05	157,50 \pm 4,57 P <0,002 P ₁ >0,05
	24	63,33 \pm 2,36 P >0,05 P ₁ <0,02	0,386 \pm 0,025 P <0,02 P ₁ >0,05	167,83 \pm 2,40 P <0,01 P ₁ >0,01
Phytin	1	66,83 \pm 3,56 P >0,05 P ₁ <0,05	0,453 \pm 0,047 P <0,02 P ₁ >0,05	147,67 \pm 3,22 P <0,001 P ₁ <0,05
	6	62,16 \pm 1,94 P >0,05 P ₁ <0,01	0,412 \pm 0,019 P <0,01 P ₁ >0,05	168,17 \pm 3,65 P <0,01 P ₁ <0,05
	24	61,06 \pm 2,07 P >0,05 P ₁ <0,01	0,341 \pm 0,014 P <0,05 P ₁ <0,01	175,16 \pm 3,08 P <0,01 P ₁ <0,05

Note: P – statistically significant in comparison with intact,

P₁ - statistically significant in comparison with the control, corresponding hour of the study

It is known that insufficient oxygen supply to organs and tissues is accompanied by a significant metabolic disorder, which leads to the development of endogenous intoxication syndrome (ESI), in which under-oxidized metabolic products, especially carbohydrate, protein and lipid, have a toxic effect. The elimination of hypoxia or the development of resistance to oxygen starvation, as a rule, should prevent the development of intoxication of the body. Proceeding from this, it was of great interest to study the effect of antihypoxants on the development of ESI. Later, we studied in a comparative aspect the effect of glisimed, phytin and piracetam on ESI indicators such as sorption capacity of erythrocytes (SCE), medium molecular weight peptides (MMP) and life expectancy of paramecium (LEP).

Analysis of the obtained results of experimental studies

showed that in the initial post-hypoxic period (after 1 hour) the serum of animals' blood contains substances that cause an increase in SCE by 49.0%, against the background of an increase in the level of MMP by 113.0%. All this led to a reduction in LEP to 130.83 \pm 4.71 seconds, against 213.17 \pm 7.44 seconds in the control. The obtained results with an increase of the observation period up to 6-24 hours did not undergo statistically significant changes.

Consequently, normobaric hypoxia is accompanied by significant changes in metabolism of substances leading to the accumulation of toxic metabolic products in the body, which is expressed in the development of ESI.

The classic representative of antihypoxant drugs - piracetam reduced the degree of endogenous intoxication. So, in the first hours of the post-hypoxic period, the ESI in

animals which treated with piracetam preventively was low by 21.1%, and the MMP by 6.1%, compared with the control animals. At the same time, LEP increased by only 7.4%. It can be seen from the data in table 2 that these indicators remained without significant changes in the subsequent hours of observation. Similar in direction, but somewhat significant results were obtained in preventively phytin received animals. Thus, the decreasing of ESI and the level of MMP after 1 hour of the post-hypoxic period were 23.8% and 16.9%, and after 6 hours - 24.5% and 16.8% and after 24 hours - 22.5% and 25.1%, respectively. On this background, LEP increased by 13.0%, 16.5% and 9.2%, respectively, during the indicated observation periods. It can be seen from the data in table 2 that the values of the studied indexes in animals preventively receiving glisimed were more positive. Thus, the decreasing of ESI and the level of MMP in the first hour of the post-hypoxic period was 26.8% and 14.7%, respectively, and the increasing of LEP was 23.1%. A more significant decrease in the level of MMP (by 20.6-32.9%) on the background of a clear decreasing of ESI (by 25.1-23.7%) and prolongation of the LEP (by 21.0-14.5%) were determined in subsequent periods of observation.

Therefore, the studied antihypoxants significantly reduce the degree of ESI. In this regard, there are some advantages of glisimed compared to other medicines.

An analysis of the results of the conducted studies allows us to conclude that the on basis of the antihypoxant effect of glisimed lies the elimination of carbohydrate metabolism disorders, which cause a decrease in the degree of endogenous intoxication which is an important factor aggravating the course of the pathological process.

4. Conclusions

1. The post-hypoxic period is accompanied by expressed and stable disturbances of carbohydrate metabolism and the development of endogenous intoxication syndrome.
2. Glisimed eliminates carbohydrate metabolism disorders a greater extent than piracetam and phytin, which characterizes by a decreasing of the level of glucose, pyruvic and lactic acid in the posthypoxic period.
3. A high degree of endotoxemia is noted in animals undergoing hypoxia, and the preventive use of antihypoxants significantly suppresses the degree of intoxication. In this regard, glisimed is somewhat superior in its pharmacological activity to piracetam and phytin.

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