PHARMACOLOGY AND TOXICOLOGY

Stress-Inducing Effect of Hypoxia of Different Origin and Its Correction with *Inula Helenium L.* Tincture

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In order to evaluate the severity of stress, the effects of single and repeated exposure to hypoxia of different origin (hemic, tissue, and circulatory) on the classical parameters of the Selye triad, cytological characteristics of lymphoid organs, and karyometric parameters (nucleus diameters) were studied in all adrenal zones, and analysis of correlations was carried out. The corrective effect of *Inula helenium* L. tincture manifested in prolongation of the lifespan and normalization of somatic and morphological parameters.

Key Words: *hypoxic exposure; karyometric analysis of adrenals; analysis of correlations*

Acute hypoxic exposure results in the development of a typical complex of changes corresponding to the anxiety stage of the general adaptation syndrome [1]. It develops in various pathologies [8]. In all cases oxygen delivery to tissues decreases to a level insufficient for maintaining cell metabolism and function. Multicomponent hypoxic injuries and phasic pattern of disturbances in the energy system prompt creation of combined drugs with a wide spectrum of antihypoxic effects, which would arrest the development of metabolic disorders in hypoxia at different levels.

We previously demonstrated a stress-protective effect of *Inula helenium L.* preparations on different models of extreme exposures [5,8]. Here we studied their effects on the weight, cell composition, and karyometric characteristics of organs playing an important role in the realization of stress mechanisms (adrenal glands, thymus, spleen) in hypoxia of different origin, and the formation of the defense and adaptive reactions to hypoxic exposure.

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MATERIALS AND METHODS

Experiments were carried out on 144 random-bred female mice (20-22 g). The animals (conventional inbred mice) were obtained from the Collection Fund of Laboratory of Experimental Biomedical Modeling, Tomsk Research Center. Tincture of *Inula helenium L.* stems (1:10) represents a complex of substances extracted from fragmented plant stems with 40% ethanol and contains 1.63% flavonoids. Dry residue is at least 2.25%. *Inula helenium L.* stems contain sequiterpenoids, phenolcarbonic acids, flavonoids, coumarins, lipids, and carotenoids [6].

The following experimental models of hypoxia were used: single hemic hypoxia (single intraperitoneal injection of 180 mg/kg sodium nitrite), repeated hemic hypoxia (two daily subcutaneous injections of 20 mg/kg sodium nitrite for 4 days and a lethal dose of the hypoxant intraperitoneally on day 5), single tissue hypoxia (25 mg/kg sodium nitroprusside intraperitoneally), repeated tissue hypoxia (daily intraperitoneal injections of 1 mg/kg sodium nitroprusside for 4 days and 25 mg/kg on day 5), single circulatory hypoxia (60 mg/kg sodium fluoride [11]), repeated

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circulatory hypoxia (10 mg/kg daily for 4 days and 60 mg/kg on day 5 [12]). Tincture of *Inula helenium L.* stems was injected as a preventive course for 5 days and 1 h before the hypoxic exposure. The weights of the spleen, thymus, adrenals was determined, hemorrhagic foci in the gastric mucosa were counted, and the depth of stress was evaluated on the base of these data [4]. The diameter of cell nuclei was measured using an ocular micrometer. For morphometry, the sections of the adrenal glands were stained with hematoxylin and eosin. Smears of the lymphoid organs were prepared and the cytograms were analyzed routinely [2,7].

The results were processed using Student's *t* test and nonparametric Wilcoxon—Mann—Whitney test. Correlation analysis was carried out using Pierson (parametric) coefficient and Spearman (nonparametric) ranked coefficient [10].

RESULTS

Individual and species survival is a general criterion of adaptation [3]. The life-span of animals treated with *Inula helenium* L. tincture increased by 35% (compared to controls) after single hemic hypoxia and by 76% after repeated hypoxia (Table 1). The stress-protective effect of the phytopreparation (difference from the control was 5 points) was realized through prevention of adrenal hypertrophy and splenic involution. Single hemic hypoxia leads to a significant increase in the diameters of the nuclei in the adrenal medulla and zona reticularis in comparison with intact controls (Table 2). Repeated exposure led to a more pronounced increase in the medullary zone nuclei. Injection of the phytopreparation eliminated cell nuclei hyperplasia in the adrenal medulla and decreased the diameter of the zona reticularis nuclei (Table 2). Analysis of splenograms showed that treatment with *Inula helenium L.* tincture removed lymphopenia of minor lymphocytes and erythrocytosis caused by hypoxic exposure, reduced the counts of monocytes and macrophages to the baseline level, and increased the count of plasma cells compared to repeated exposure and intact control. After acute exposure the count of plasma cells in the thymogram increased, while repeated injections of sodium nitrite decreased this parameter to the baseline level. Treatment with *Inula helenium L.* tincture 5.6-11.6-fold increased the count of these cells in cytological preparation.

Treatment with *Inula helenium L.* tincture prolonged the life-span of animals exposed to single and repeated tissue hypoxia by 20 and 29%, respectively. The stress-protective effect was 5 points due to moderation of adrenal hypertrophy. Single injection of sodium nitroprusside to animals led to enlargement of the nuclei in all adrenal zones except the medullary zone, where just a trend to nucleus enlargement was observed. Repeated injection of the hypoxant followed by the treatment with *Inula helenium L.* tincture virtually did not change these characteristics (Table 2). The use of *Inula helenium L.* preparation abolished the inhibitory effect of repeated hypoxic exposure on lymphocyte maturation, reduced the increased percentage of macrophages, and increased the count of plasma cells. *Inula helenium L.* tincture increased the percentage of minor lymphocyte (1.2 times) in cytological preparations of the thymus suppressed under the effect of repeated hypoxia.

The life-span of animals treated with *Inula helenium* L. tincture after single intoxication with sodium fluoride was prolonged by 59%, of those exposed to repeated hypoxia by 29%. The severity of stress decreased by 5 and 4 points, respectively, compared to the control due to the protective effect of the preparation on the gastric mucosa and the weights of the thymus and spleen. The treatment reduced the diameters of nuclei in the zona fassiculata and adrenal medula and prevented nuclear hyperplasia in the zona reticularis, induced by repeated hypoxia (Table 2). Repeated intoxication with the fluoride compound decreased the content of minor lymphocytes and increased the counts of plasma, macrophageal, erythroid, and apoptotic cells in cytological preparation of the spleen. Treatment with the phytopreparation led to the development of a clear-cut trend to recovery. The thymogram after treatment with *Inula helenium L.* tinc-

TABLE 1. Effect of *Inula Helenium L.* Tincture on Life Span of Animals Exposed to Hypoxia of Different Origin ($X \pm m$, n=6-10)

Group	Life span, min					
	hemic hypoxia		tissue hypoxia		circulatory hypoxia	
	single	repeated	single	repeated	single	repeated
Control	51.3±5.1	16.8±0.6	13.1±0.9	11.3±0.5	105.9±25.3	161.7±22.1
Inula helenium L. tincture (2.5 ml/kg)	59.6±4.9*	29.6±5.6*	15.7±0.2*	14.6±1.3*	168.4±23.6*	209.4±15.2*

Note. *p<0.05 compared to the control.

TABLE 2. Effect of *Inula Helenium* L. Stem Tincture on the Diameter (μ) of Cell Nuclei in Different Adrenal Zones under Conditions of Hypoxia of Different Origin ($X \pm m$, n=6-10)

Adrenal zone		Intact	Control 1	Control 2	*Inula Helenium* L. stem tincture
Hemic hypoxia	zona glomerulosa	6.29±0.09	6.86±0.13	6.62±0.10	6.67±0.10
	zona fasciculata	6.29±0.06	6.58±0.12	6.96±0.10*	6.60±0.14
	zona reticularis	6.31±0.09	7.10±0.24*	7.46±0.18*	6.79±0.13*+
	adrenal medulla	7.61±0.15	8.52±0.08*+	9.07±0.24*	6.67±0.36*+
Tissue hypoxia	zona glomerulosa	6.29±0.09	6.94±0.21*	6.72±0.21	6.72±0.04
	zona fasciculata	6.29±0.06	6.72±0.08*	6.84±0.15	6.67±0.09
	zona reticularis	6.31±0.09	6.94±0.19*	6.75±0.27	7.20±0.10
	adrenal medulla	7.61±0.15	8.11±0.23	8.10±0.37	8.21±0.14
Circulatory hypoxia	zona glomerulosa	6.29±0.09	6.58±0.24	6.41±0.03	6.12±0.09+
	zona fasciculata	6.29±0.06	6.55±0.16	6.65±0.19	6.31±0.05
	zona reticularis	6.31±0.09	6.55±0.14	6.89±0.13*	6.41±0.14
	adrenal medulla	7.61±0.15	7.42±0.28	7.85±0.29	7.18±0.31*+

Note. Control 1: single hypoxia exposure (unadapted animals); control 2: repeated exposure (adapted animals). $p<0.05$ *compared to intact animals, +compared to control 2.

ture revealed a decrease in erythroid reaction, which was enhanced after injection of sodium fluoride, and increase in the number of plasma cells in comparison with the baseline level.

Qualitative structure of interactions between the parameters of adaptation to hypoxic exposure was evaluated by the analysis of correlations. In our studies the life-span prolongation in hypoxia of different origin (which was regarded as a favorable course of adaptation) was combined with increased correlations between thymus parameters (organ weight and thymogram); they quantitatively predominated over splenic parameters (organ weight and splenogram) and adrenal parameters (organ weight and karyometric parameters in different adrenal zones). It seems that adaptation develops more rapidly under conditions of hypoxic training (repeated exposure) and treatment with *Inula helenium L.* tincture, when the thymus is "actively" involved. This ratio is characteristic of intact animals. The number of significant correlations for all thymus parameters increased under the effect of *Inula helenium L.* stem tincture, while the total number of significant relationships decreased, that is, the phytopreparation increased autonomy and autoregulation.

Thus, the development of hypoxia of different origin is characterized by initial stress reaction and a complex of shifts. The effect of *Inula helenium L.* is aimed primarily at preserving hormonal homeostasis. Studies at the cell level showed that the preparation changed the diameters of cell nuclei in the adrenal medulla (catecholamine synthesis), zona reticularis (androgen synthesis), zona glomerulosa (synthesis of mineralocorticoids) during hypoxia. It can be hypothesized that *Inula helenium L.* tincture also corrected secretion of these hormones. The regulatory effect of the phytopreparation was also detected in the analysis of cytological preparations of other "classical" stress organs (spleen and thymus), which confirms their involvement in the realization of the immune process developing as a result of combined activities of the bone marrow, thymus, spleen, and lymph nodes. The positive effect of *Inula helenium L.* phytopreparation on the formation of the defense and adaptive reaction to hypoxia was confirmed by the results of the analysis of correlations.

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