Sodium Nitroprusside-Induced Tissue Hypoxia and Its Correction with Plant Preparations

S. G. Aksinenko, T. N. Povet'eva, N. V. Provalova, K. L. Zelenskaya, Yu. V. Nesterova, A. V. Gorbacheva, E. V. Popova, Yu. G. Nagornyak, I. V. Shilova, and N. I. Suslov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Supplement 1, pp. 49-53, January, 2007
Original article submitted November 11, 2006

Tissue hypoxia caused by acute or repeated administration of sodium nitroprusside to experimental mice induced changes in hemopoietic and lymphoid organs. Isolated flavonoids and alkaloids as well as complex plant preparations abolished the negative consequences caused by the hypoxant and prolonged the lifespan of experimental animals.

Key Words: *tissue hypoxia; sodium nitroprusside; hemopoietic and lymphoid organs; plant preparations*

Tissue hypoxia is characterized by disturbed utilization of oxygen for biooxidation by cells and reduced efficiency of entrapping free energy released during biooxidation, which is usually associated with uncoupling of oxidation and phosphorylation [2]. The hypoergic state activates free-radical processes and impairs antioxidant defense. LPO products damage biomembranes (including mitochondrial membanes) and aggravate energy metabolism disturbances, *i.e.* a vicious circle is thus forming. It is known that sodium nitroprusside (SN) is a donator of nitric oxide (NO) and in high concentrations induces tissue hypoxia [11]. Endogenous NO is involved in various physiological and cell responses, but can be a pathological factor in neurogenic pathologies of CNS, ischemia, brain stroke, convulsive disorders [9].

We previously demonstrated stress-inducing effects of hypoxia of various geneses and the possibility of its correction with plant-derived preparations [6]. It is well known that many natural bioactive substances (*e.g.* flavonoids and alkaloids) produce an antioxidant effect and improve the resistance to hypoxia [10]. Plant preparations and bioactive substances isolated from plants can be used as correctors in acute and repeated tissue hypoxia, which was the subject of the present study.

Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences

MATERIALS AND METHODS

The experiments were carried out on 120 male and female outbred mice and 34 female CBA mice obtained from the Department of Biomedical Modeling, Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences. The animals were kept in accordance with European Convention on Protection of Vertebrate Animals Used in Experimental and Scientific Purposes (Strasbourg, 1986). Acute tissue hypoxia was modeled in mice by intraperitoneal injection of 25 mg/kg SN [7]. Plant preparations were administered *per os* for 5 days (prophylactic course), the last dose was given 1 h before hypoxic exposure. The animals received 0.6 and 2.5 mg/kg delphinium (*Delphinium elatum*) tincture, 0.12 and 0.25 delphinium extract, 0.05 and 1.50 mg/kg delphinium alkaloids, 2 ml/kg tincture from poison hemlock (*Conium maculatum*) dry herb, 2 ml/kg tincture from wet poison hemlock herb, 1 ml/kg tincture from dry poison hemlock inflorescences, 2 ml/kg tincture from fresh poison hemlock inflor-

0007-4888/07/Supp-042

escences, 25 mg/kg ethylacetate fraction from poison hemlock inflorescences, and 25 mg/kg ethylacetate fraction from poison hemlock herb. Repeated tissue hypoxia was modeled by intraperitoneal injection of 1 mg/kg SN for 4 days and 25 mg/kg SN on day 5 [6]. The plant preparations in this case were administered 1 h before hypoxant injection in the following doses: 5 ml/kg ethanol extract from overground parts of meadowsweet (*Filipendula ulmaria*), 5 ml/kg osier (*Salix viminalis*) leaves, and 100 mg/kg Alfredia cernua extract. All tinctures and extracts were standardized by dry residue [4].

Control animals received the solvent according to the same scheme. α-Tocopherol acetate in a dose of 100 mg/kg was used as the reference drug [8].

After 5-folg hypoxic exposure, animal lifespan, weights of the spleen, thymus, and adrenal glands, and the number of hemorrhages on the gastric mucosa (GM) were evaluated. The total numbers of myelokaryocytes, splenocytes, thymocytes, and peripheral blood leukocytes were determined [3].

The data were processed statistically using Student's *t* test, and nonparametric Wilcoxon—Mann—Whitney *U* test [5].

RESULTS

Control mice died 9-11 min after single injection of SN (Fig. 1). Plant preparations reduced the toxic effects of the hypoxant. In animals receiving tinctures of wet and dry poison hemlock herb, the lifespan increased by 31-38% compared to the control. Administration of ethylacetate fraction of poison hemlock flavonoids also increased this parameter (fraction from inflorescences by 49% and from herb by 46%). The most pronounced antihypoxic effect was produced by tincture of dry and wet poison hemlock inflorescences (lifespan prolongation by 55 and 60%, respectively). Administration of α-tocopherol acetate prolonged animal lifespan by 38% compared to the control.

Administration of delphinium extracts also produced an antihypoxic effect (Fig. 1). Thus, the lifespan of mice receiving delphinium tincture in doses of 0.6 and 2.5 ml/kg increased by 114 and 149%, respectively. Administration of delphinium extract also prolonged lifespan by 98 and 93%. Prophylactic course of delphinium alkaloids increased lifespan of experimental animals by 69 and 80% compared to the control, while administration of the reference preparation increased this parameter by 135%.

Comparative analysis showed that even single hypoxic exposure produced typical somatic changes in the weight of stress-marker organs (Fig. 2). Acute hypoxic trauma induced involution of the spleen and thymus (their weight decreased by 33 and 24%, respectively) and hypertrophy of the adrenal glands (their weight surpassed that in intact controls by 15%). Hemorrhages and erosions appeared in GM. Chronic hypoxic exposure induced more pronounced decrease in thymus weight, more pronounced increase in the weight of the adrenal glands, and greater number of ulcerative lesions in GM, although these differences were not significant. Administration of *Alfredia cernua* extract had a protective effect on the stress-marker organs: weight of the spleen and adrenal glands returned to the initial values, weight of the thymus increased by

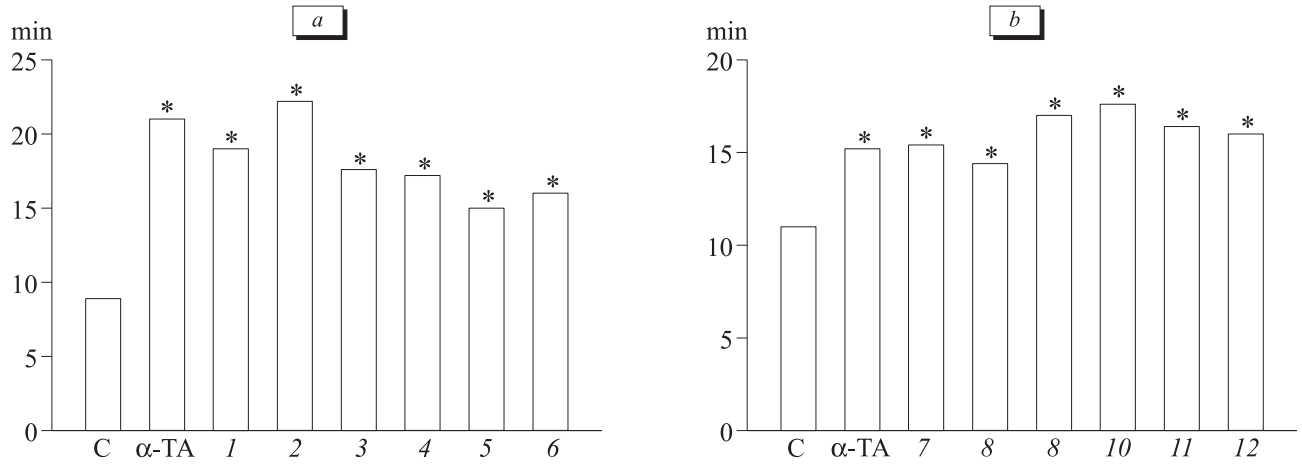

Fig. 1. Effects of delphinium (*a*) and poison hemlock (*b*) on lifespan of animals under conditions of acute tissue hypoxia. The animals received *Delphinium elatum* tincture in doses of 0.6 (*1*) and 2.5 ml/kg (*2*), extract in doses of 0.12 (*3*) and 0.25 mg/kg (*4*), or alkaloids in doses of 0.05 (*5*) and 1.5 mg/kg (*6*); tinctures from poison hemlock dry herb (*7*), wet herb (*8*), dry inflorescences (*9*), fresh inflorescences (*10*), ethylacetate fraction from inflorescences (*11*), and ethylacetate fraction from herb (*12*). C: control; α-TA: α-tocopherol acetate. **p*<0.05 compared to the control.

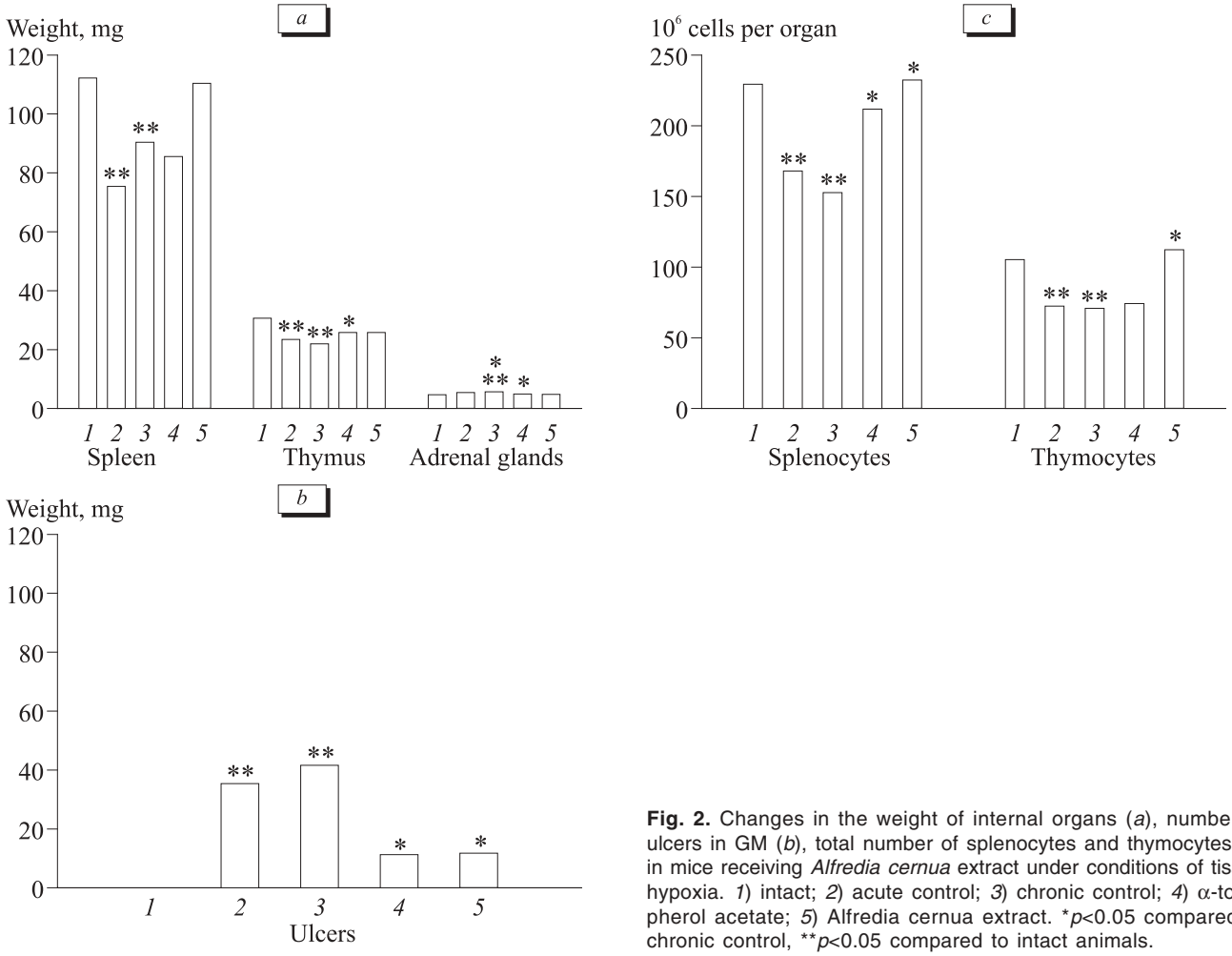

Fig. 2. Changes in the weight of internal organs (*a*), number of ulcers in GM (*b*), total number of splenocytes and thymocytes (*c*) in mice receiving *Alfredia cernua* extract under conditions of tissue hypoxia. *1*) intact; *2*) acute control; *3*) chronic control; *4*) α-tocopherol acetate; *5*) Alfredia cernua extract. **p*<0.05 compared to chronic control, ***p*<0.05 compared to intact animals.

17%, the number of hemorrhages in GM decreased by 3.6 times. α-Tocopherol acetate prevented adrenal gland hypertrophy and produced a protective effect on the thymus and GM, but the weight of the spleen remained at the control level.

The development of hypoxia reduced the total number of splenocytes and thymocytes by 27% and 31%, respectively. After chronic exposure the cell depletion of lymphoid organs was more pronounced, but insignificant. The therapeutic course of *Alfredia cernua* extract against the background of hypoxia normalized the total cellularity of the spleen and thymus, while administration of α-tocopherol acetate increased only spleen cellularity (Fig. 2).

Hypoxic trauma decreased the total number of myelokaryocytes in control animals by 24.4% due to pronounced decrease in the number of lymphocytes in the bone marrow compared to healthy mice (Fig. 3). Moreover, the total number of peripheral blood leukocytes also decreased in the control group.

The total number of myelokaryocytes in mice receiving course treatment with extracts from meadowsweet and osier leaves or reference drug surpassed the control values by 1.1-1.2 times. The number of bone marrow nuclears in these groups was preserved primarily due to the number of immature neutrophil granulocytes and erythroid cells. The total number of peripheral blood leukocytes in these mice did not significantly differ from that in healthy animals and 1.1-1.3 fold surpassed the corresponding value in the control group (Fig. 3).

Our experiments showed that hypoxia caused by single or repeated administration of SN induced complex changes in the bone marrow and lymphoid organs. Isolated flavonoids and alkaloids as well as complex plant preparations abolished the negative consequences caused by the hypoxant.

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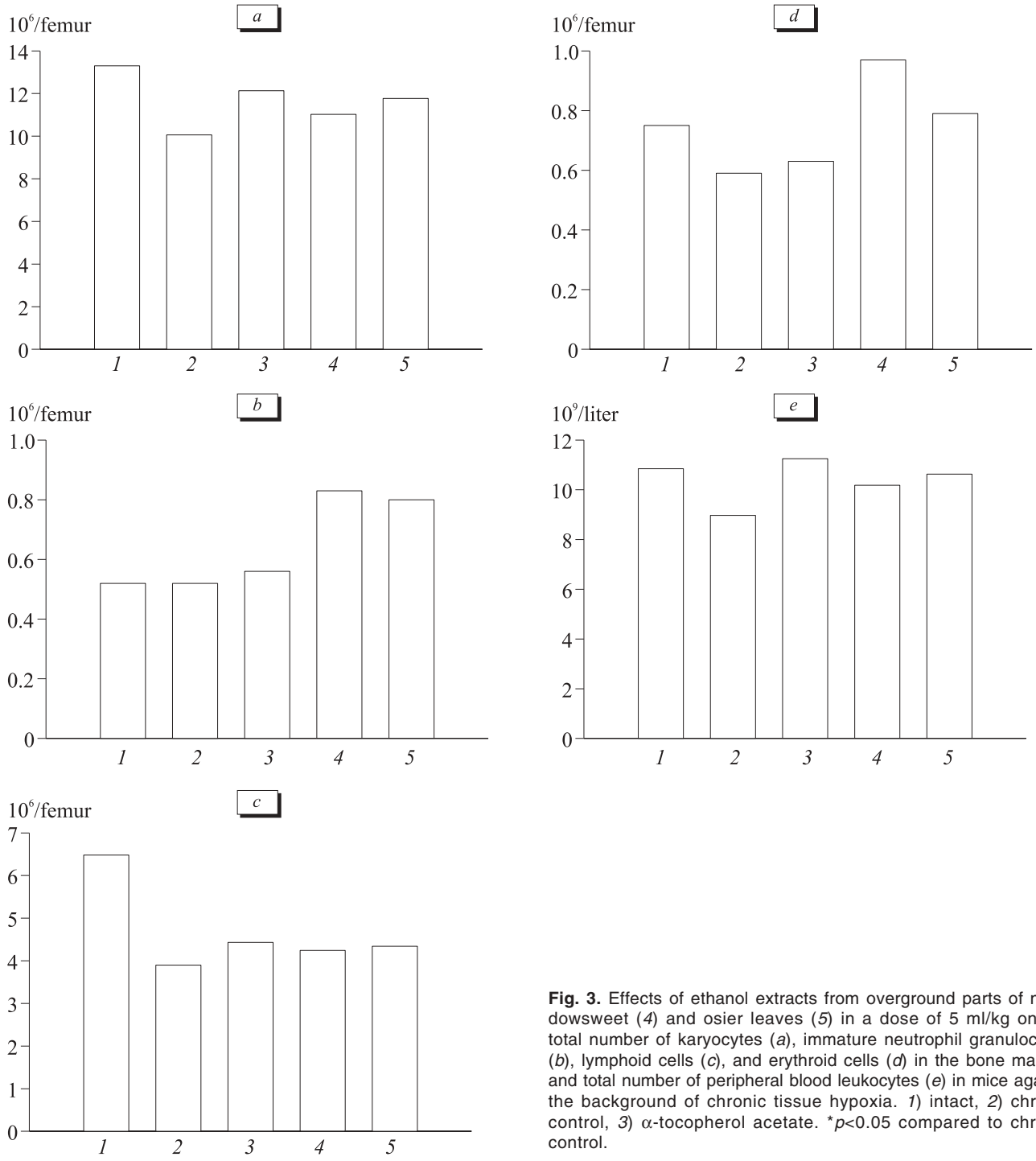

Fig. 3. Effects of ethanol extracts from overground parts of meadowsweet (*4*) and osier leaves (*5*) in a dose of 5 ml/kg on the total number of karyocytes (*a*), immature neutrophil granulocytes (*b*), lymphoid cells (*c*), and erythroid cells (*d*) in the bone marrow and total number of peripheral blood leukocytes (*e*) in mice against the background of chronic tissue hypoxia. *1*) intact, *2*) chronic control, *3*) α-tocopherol acetate. **p*<0.05 compared to chronic control.

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