

EFFECTS OF NONSYMMETRICAL DIMETHYLHYDRAZINE AT THE PROTEIN DEFICIENCY IN DIET ON THE GROWING ANIMALS

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The purpose of the present research NDMH was influence on a condition of biochemical processes in blood plasma of the growing animals receiving a diet with deficiency of protein. Effects of nonsymmetrical dimethylhydrazine at the protein deficiency in diet of the growing animals has been represented by superfluous accumulation of the oxidized proteins and infringement of the structure-forming properties of blood plasma in growing animals.

Our researches has shown, that the expressed infringements of an oxidative metabolism, increase of extracellular nucleic acids in blood and changes of the blood plasma teziograms morphotypes are fixed at growing animals for 30 days after unitary injection of nonsymmetrical dimethylhydrazine (NDMH) [1].

It is shown, that the alimentary factor has essential influence on reactions of an organism of animals at NDMH injection [2-6].

NDMH influence on a condition of biochemical processes in blood plasma of the growing animals receiving a diet with deficiency of protein is not studied practically that has served as the purpose of the present research.

Research materials and methods

Experiment has been carried out on 60 white not purebred infant rats-weanling of both sexes with initial weight of body 50 g, divided into 3 groups (control, comparison group and basic group). NDMH has been unitary injected in a dose 5 mg/kg intraperitoneally to animals of the comparison group and basic group. Experiment term lasts 30 days. Animals of control group and comparison group received the general vivarium diet during all experiment. Animals of the basic group has been kept on an isohigh-calorie semisynthetic diet during 30 days.

The structure of an isohigh-calorie semisynthetic diet was presented by protein of gluten (8 %), deficient on methionine, lysine and threonine, lard (15 %), carbohy-

drates (68 %), a mineral component (4 %), a vitamin mix (4 %), small wood sawdust (1 %). The carbohydrate component is presented by granulated sugar (10 %) and potato starch (58 %). Mineral and vitamin components was made according to recommendations [7]. Animals received forage and water without restrictions. The forage rests were considered.

Animals has been deduced from experiment by a method of incomplete decapitation under an easy ether narcosis according to the international recommendations [8].

Morphology of blood plasma has been defined by a method wedge-shaped dehydro-tation. A plasma drop has been placed on a fat-free surface and dried up at a room temperature. The dry film-facies, reflecting intermolecular interactions in blood plasma, was formed after water evaporation [9].

Degree of spontaneous and oxidation metal-catalyzed protein oxidation (MCPO) has been defined in blood plasma. The method of an estimation of the protein oxidative modification (POM) has been based on reaction of interaction of oxidized aminoacidic residues with 2,4-dinitrophenylhydrazine with formation of ketone dinitrophenylhydrazones (KDNPH) and aldehyde dinitrophenylhydrazones (ADNPH) of the basic and neutral character. Protein oxidative modification has been initiated by Phenton medium. Degree of protein oxidative modification was expressed in units of the optical density, related on 1 ml

of plasma [10]. Results of biochemical researches has been processed according to the method of Mann-Whitney [11].

Results of research

The analysis of the blood plasma phascias morphotypes in infant rats-weanling of the comparison group has revealed presence of the following general trends: partial loss of an intermediate zone, occurrence of chaotic type of cracking and decrease of the concretion quantity. Similar changes of the phascias morphotypes are markers of system changes of the structure-forming properties of blood plasma in growing animals for 30 days after unitary NDMH injection [1].

Morphotypes of blood plasma in growing animals of the basic group are character-

ised by an infringement of the phascias formation zonality, loss of symmetry and equi-radial cracking, formation of amorphous areas in the central zones of phascias, the general decrease of the concretion quantity and occurrence of the concretion atypical forms.

However there are also differences in morphotypes of the blood plasma teziograms of females and males. Elements of three-zone structure (fig. 1) remain in 60 % of the blood plasma teziograms of females-weanling whereas phascias formation zonality has not been expressed, or was absent in 40 % of teziograms. Generated concretions were characterized by the small sizes.

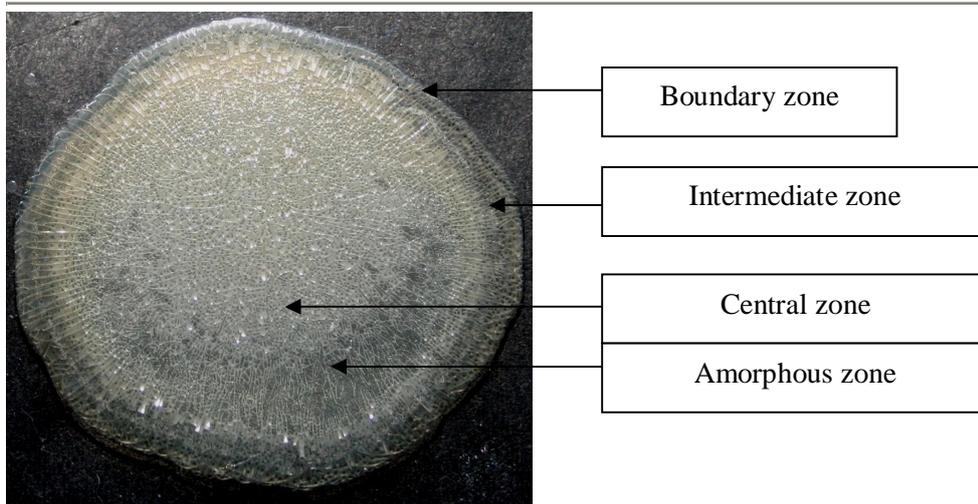


Fig. 1. Teziograms of females-weanling at basic group

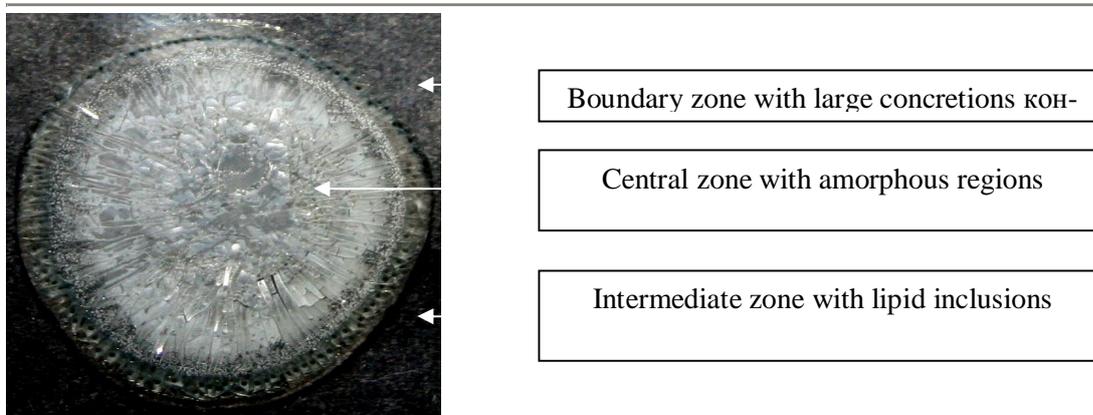


Fig. 2. Teziograms of males-weanling at basic group

Large amorphous areas in the central zone were observed in 20 % of blood plasma teziograms of male. Accurate large concretions were fixed in 60 % of teziograms. We has noted formation of the concretions with different sizes (large and small - dot) and different configuration (round, extended, rod-like) within one phascia.

Similar changes of the phascias morphotypes are markers of not only system, but also subsystem changes of the structure-forming properties of blood plasma in growing animals of the basic group with deficiency of protein in a diet.

Strengthening of the protein modification oxidation can serve as one of the possible causes of infringement of the blood plasma phascias morphotypes in rat-weanlings of basic group. Definition of of the spontaneous POM catabolites has shown, that at growing animals of the basic group the contents of dinitrophenylhydrazones of basic and neutral character authentically differed from those of the control.

We has revealed reliable increase in the contents of dinitrophenylhydrazones of basic and neutral character in blood plasma of rats at the basic group in comparison with those of animals of the 2nd group.

Table 1. Influence of NDMH unitary injection on indicators of spontaneous protein oxidative modification in blood plasma of the growing animals, kept on a diet with deficiency of protein, (M+m)

Groups of animals	Sample	KDNPH of neutral character, reference units/ml	ADNPH of neutral character, reference units/ml	KDNPH of basic character, reference units/ml	ADNPH of basic character, reference units/ml
Control	Σ (n=9)	4,88±0,51	5,22±0,45	2,75±0,49	0,439±0,054
	♀ (n=5)	4,82±0,63	5,18±0,54	2,65±0,54	0,434±0,061
	♂ (n=4)	4,96±0,37	5,27±0,39	2,87±0,46	0,445±0,052
Comparison group (NDMH unitary injection)	Σ (n=30)	7,16±0,73 *	8,01±0,71 *	5,91±0,64 *	0,974±0,088 *
	♀ (n=15)	6,95±0,70 *	7,84±0,71 *	5,73±0,62 *	0,961±0,093 *
	♂ (n=15)	7,38±0,70 *	8,18±0,69 *	6,09±0,62 *	0,987±0,083 *
Basic group (NDMH unitary injection +protein-deficient diet)	Σ (n=20)	8,99±0,31 *#	9,86±0,38 *#	7,50±0,35 *#	1,383±0,054 *#
	♀ (n=10)	9,04±0,33 *#	9,91±0,41 *#	7,47±0,35 *#	1,381±0,059 *#
	♂ (n=10)	8,96±0,31 *#	9,81±0,36 *#	7,53±0,37 *#	1,383±0,056 *#

Notes: * - reliability of indicators in relation to the control (p<0,01);
- reliability of distinctions of the basic group in relation to comparison group (p <0,01)

We has revealed the increase of the blood plasma protein sensitivity in animals of the experimental group to action of prooxidizers is revealed. It is documented by reliable increase of the contents of metal-

catalyzed POM catabolites. Level of catabolites formed at addition of Phenton medium not only reliably exceeded those of the control, but also of comparison groups.

Table 2. Influence of NDMH unitary injection on indicators of metal-catalyzed protein oxidative modification in blood plasma of the growing animals, kept on a diet with deficiency of protein, ($M \pm m$)

Groups of animals	Sample	KDNPH of neutral character, reference units/ml	ADNPH of neutral character, reference units/ml	KDNPH of basic character, reference units/ml	ADNPH of basic character, reference units/ml
Control	Σ (n=9)	9,25±1,69	10,57±1,74	4,79±0,79	0,999±0,150
	♀ (n=5)	9,12±1,75	10,58±2,15	4,82±0,76	0,984±0,158
	♂ (n=4)	9,42±1,87	10,55±1,41	4,75±0,94	1,017±0,162
Comparison group (NDMH unitary injection)	Σ (n=30)	17,65±0,77 *	18,77±0,74 *	8,47±0,53 *	1,929±0,109 *
	♀ (n=15)	17,43±0,73 *	18,60±0,78 *	8,42±0,49 *	1,909±0,121 *
	♂ (n=15)	17,87±0,77 *	18,93±0,68 *	8,53±0,58 *	1,950±0,095 *
Basic group (NDMH unitary injection +protein-deficient diet)	Σ (n=20)	19,48±0,62 *#	20,22±0,74 *#	10,97±0,87 *#	2,435±0,319 *#
	♀ (n=10)	19,30±0,57 *#	20,01±0,54 *#	10,77±0,77 *#	2,370±0,360 *#
	♂ (n=10)	19,67±0,65 *#	20,44±0,87 *#	11,18±0,95 *#	2,500±0,277 *#

Notes: * - reliability of indicators in relation to the control ($p < 0,01$);
- reliability of distinctions of the basic group in relation to comparison group ($p < 0,01$)

Prooxidant effect of NDMH is the reasons of superfluous accumulation of the oxidized proteins in blood plasma of growing animals [1]; oxidative stress is also developed owing to deficiency of protein in a diet [12]. It determines infringement of the structure-forming properties of blood plasma in growing animals. Thus, deficiency of protein in a diet of growing animals appreciably aggravates detrimental effect of NDMH.

References:

1. Condition of oxidative metabolism and crystal-forming characteristic of blood of experimental animals at an intoxication of nonsymmetrical dimethylhydrazine / I.R. Kulmagambetov, L.Ye. Muravleva, V.V. Koikov, Yu.E. Abdrahmanova et. al. // Biomedical chemistry 2007, V. 53 (3), P. 276-284
2. Effect of dietary carbohydrates on the growth of dysplastic crypt foci in the colon of rats treated with 1,2-dimethylhydrazine / Caderni G, Bianchini F, Mancina A, Spagnesi MT, Dolara P. // Cancer Res. 1992 Aug 1;52(15):4291-2.
3. Latham P, Lund EK, Johnson IT. Dietary n-3 PUFA increases the apoptotic response to 1,2-dimethylhydrazine, reduces mitosis and suppresses the induction of carcinogenesis in the rat colon // Carcinogenesis. 1999 Apr;20(4):645-50
4. Dietary protein and chronic toxicity of 1,2-dimethylhydrazine fed to mice /Visek WJ, Clinton SK, Imrey PB, Thursh DR, Truex CR, Alster JM, Anderson PA, Mabry FJ, Nandkumar S, Simon J. // J Toxicol Environ Health. 1991 Apr;32(4):383-413
5. Effect of heat processing and of vegetables and fruit in human diets on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats / Alink GM, Kuiper HA, Hollanders VM, Koeman JH. // Carcinogenesis. 1993 Mar;14(3):519-24.
6. Effects of dietary perilla oil, soybean oil and safflower oil on 7,12-dimethylbenz[a]anthracene (DMBA) and 1,2-dimethyl-hydrazine (DMH)-induced mammary gland and colon carcinogenesis in female SD rats / Hirose M, Masuda A, Ito N, Kamano K, Okuyama H. //Carcinogenesis. 1990 May;11(5):731-5
7. Methodical recommendations on biological estimation of the products of the feeding / V. Shablii, A. Ignatyev, M. Kerimova et. al. - M., 1973.- 30 p.

8. The International recommendations on undertaking physician-biological studies with use animal // WHO Chronicle – 1995 - V.39 (3).- P.3-9
9. Shabalina V.N., Shatokhina S.N. Morphology of man's biological liquids – M., 2001.– 303p.
10. Dybinina E.V. Oxidative modification of proteins in blood serum. Method of its detection // Annals of Medical Chemistry – 1995.- №1.- P. 24-27.
11. Mann H.B., Whitney D.R. On a test of whether one of two random variables is stochastically larger than the other. // Annals of Mathematical Statistics. 1947. № 18. P. 50-60.
12. Oxidative metabolism of the growing animals an the unitary injection of nonsymmetrical dimethylhydrazine in condition of little – protein diet / Muravleva L.Ye., Koikov V.V., Muratova A.Z., Abdrakhmanova Yu.E. et al. // Proceeding of the 5-th National Scientific Conference with International Participation “Reactive Oxygen Species, Nitric Oxide, Antioxidant and Human Health” – Smolensk, 2007- P. 476-478.