Effect of Nutritional Indoles on Activity of Xenobiotic Metabolism Enzymes and T-2 Toxicity in Rats

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Experiments on Wistar rats showed that feeding a ration containing 0.1% concentrate of food indoles (indole-3-carbinole and ascorbigen) for 3 weeks increased activity of phases I and II xenobiotic metabolism enzymes in the liver and intestinal mucosa and weakened the toxic effects of trichothecene T-2 mycotoxin. Activity of the key enzymes of T-2 detoxification, microsomal carboxylesterase and UDP-glucuronosyl transferase, was 1.5-2-fold higher in rats receiving T-2 toxin against the background of indole-enriched diet compared to toxin-treated rats kept on standard ration.

Key Words: *T-2 toxin; enzymes of xenobiotic metabolism; nutritional indoles*

Numerous recent studies showed the important role of minor bioactive food components in the maintenance of general status and prevention of a number of chronic diseases including tumors, cardiovascular diseases, and diabetes. Some authors regard these diseases as a manifestation of maladaptation resulting from low consumption of food components activating protective and adaptive capacities of an organism.

Natural chemopreventive compounds including food indoles and isothiocyanates are produced during hydrolysis of glucosinolates in plants of the Cruciferae family (all kinds of cabbage and radish).

Biological activity of food indoles, indole-3-carbinole, ascorbigen, and indole-3-acetonitrile, depends on their ability to activate monooxygenase system (mostly CYP1A1 and CYP1A2) and some enzymes (glutathione transferase) of phase II xenobiotic metabolism in the liver, intestine, and other organs [15]. Isothiocyanates, sulforafan, phenethyl isothiocyanate, and benzyl isothiocyanate, are potent inductors of phase II xenobiotic metabolism enzymes. In contrast to indoles, they do not affect monooxygenase activity and can competitively or by covalent binding inhibit some isoforms of cytochrome P-450, CYP1A1, CYP1A2, and CYP2E1 activating benzo(a)pyrene, aflatoxin B_1, and nitrosamines [6,9]. Experiments showed the ability of food indoles and isothiocyanates to inhibit carcinogenic effect of aflatoxin, nitrosamines, dimethylbenzanthracene, and to decrease the frequency of spontaneous tumors in rats and mice [7,11]. Epidemiological studies revealed a correlation between high consumption of vegetables of the Cruciferae family and decrease of the frequency of some hormone-dependent tumors.

The purpose of the present study was to examine the effect of food indoles on the toxicity of T-2 toxin, a member of trichothecene mycotoxins wide spread as natural contaminants of food grain [13]. T-2 toxicity was evaluated by activity of xenobiotic metabolism enzymes and lysosomal enzyme maintaining adaptive and protective processes and characterized by high sensitivity to fusareotoxins [2].

MATERIALS AND METHODS

Two groups of male Wistar rats (each comprised 20 animals) were used in the study. Group I rats received full-value semisynthetic basic ration, group II was kept on indole-enriched ration. Biologically active food additive ExPress (Enrich) containing indole-3-

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carbinole, ascorbigen, and sulforafan from broccoli was used as a source of indoles. The preparation was added to food to provide 0.1% indole content. During the last 8 days half of animals received 0.8 mg/kg T-2 toxin ($^1/_7$ LD_{50} for rats), control animals received equal volume of solvent (0.1% aqueous solution of ethanol).

The state of the animals, food consumption, and body weight were controlled daily. Damaging action of the toxin on the liver, cell membranes in liver homogenates, and cytosol fraction was evaluated by total and nonsedimented activity of lysosome enzymes: arylsulfatase A and B, β-galactosidase, and β-glucuronidase, as well as the activity of serum α-mannosidase and N-acetylglucosaminidase [1]. Activity of phase I and II enzymes participating in T-2 xenobiotic metabolism was detected in liver microsomes and small intestinal mucosa. The following enzymes were measured: total content of cytochrome P-450 and activities of benzo(a)pyrene hydroxylase [10], carboxylesterase [12], epoxydhydrolase [3], UDP-glucuronosyl transferase with p-nitrophenyl (p-NP) and 4-hydroxybiphenyl (HBP) as acceptors [5]. Glutathione transferase activity was determined in cytosol with 1-chloro-2,4-dinitrobenzene as a cofactor [8]. Specimen of the internal organs and tissues were used for histology and histochemistry. Chromatographically pure (over 98%) T-2 toxin was isolated from *Fusarium sporotrichiella 53315* culture. Statistical processing of the results was performed by the method of dispersion analysis (ANOVA) and Student's *t* test.

RESULTS

Consumption of indole-enriched diet for 3 weeks did not change animal growth, relative weight of internal organs, enzyme activity in liver lysosomes and serum, and morphology of internal organs and tissues. At the same time, activity of the examine enzymes of xenobiotic metabolism in the liver significantly surpassed the control level (Table 1). Thus, total content of cytochrome P-450 and benzo(a)pyrene oxidation rate (specific substrate CYP1A1) increased by 56 and 30%, respectively, carboxylesterase activity by 37%, while activity of phase II xenobiotic metabolic enzymes p-NP-UDP-glucuronosyl transferase, HBP-UDP-glucuronosyl transferase (1A and 2B subfamilies, respectively) and glutathione transferase, increased by 145, 40, and 76%, respectively. Significant increase in the activities of benzo(a)pyrene hydroxylase (about 2-fold) and carboxylesterase (by 55%) was revealed in the small intestinal mucosa in rats receiving indole-enriched ration (Table 1). The increase in the activity of conjugation enzymes, UDP-glucuronosyl transferases and glutathione transferase was less pronounced.

In rats receiving basic ration, T-2 toxicity was characterized by previously described clinical, biochemical, and morphological changes [2]. By the end of the experiment these animals showed decreased body weight. Relative liver weight increased by 26%, while that of the thymus decreased by 41%. Serum activity of lysosome enzymes, α-mannosidase and N-acetylglucosaminidase, decreased by 33 and 56%, respectively. Nonsedimented activity of β-galactosidase, β-glucuronidase, and arylsulfatases decreased to 53, 47, and 63% of the control level, respectively (Fig. 1, *c*). T-2 toxic action decreased cytochrome P-450 content (more than twice), inhibited carboxylesterase and benzo(a)pyrene hydroxylase activities by 30 and 26%, respectively, and reduced microsomal protein concentration (Fig. 2).

Morphological examination revealed enlargement and lipid infiltration of the liver, reduction of the thymus, and pronounced hypoplasia of lymphoid tissue.

TABLE 1. Activity of Xenobiotic Metabolic Enzymes (per mg Protein) in the Liver and Small Intestinal Mucosa in Rats Receiving Indole-Enriched Ration (*M*±*m*)

Parameter	Liver		Small intestine	
	basic ration	+indoles	basic ration	+indoles
Cytochrome P-450, nmol/mg protein	0.88±0.07	1.37±0.08*	—	—
Benzo(a)pyrene hydroxylase, fl. units/min	14.3±0.9	18.6±0.3*	0.103±0.010	0.202±0.021*
Epoxide hydrolase, nmol/min	5.4±0.3	6.37±0.14*	—	—
Carboxylesterase, μmol/min	1.83±0.09	2.50±0.12*	1.00±0.01	1.55±0.10*
UDP-glucuronosyl transferase, nmol/min				
p-NP	26.7±1.0	65.5±2.0*	4.44±0.74	5.90±1.07
HBP	22.4±1.4	31.4±1.8*	2.36±0.21	2.82±0.34
Glutathione transferase, μmol/min	0.67±0.05	1.18±0.03*	54.0±1.4	62.6±4.3

Note. *$p<0.05$ compared to basic ration.

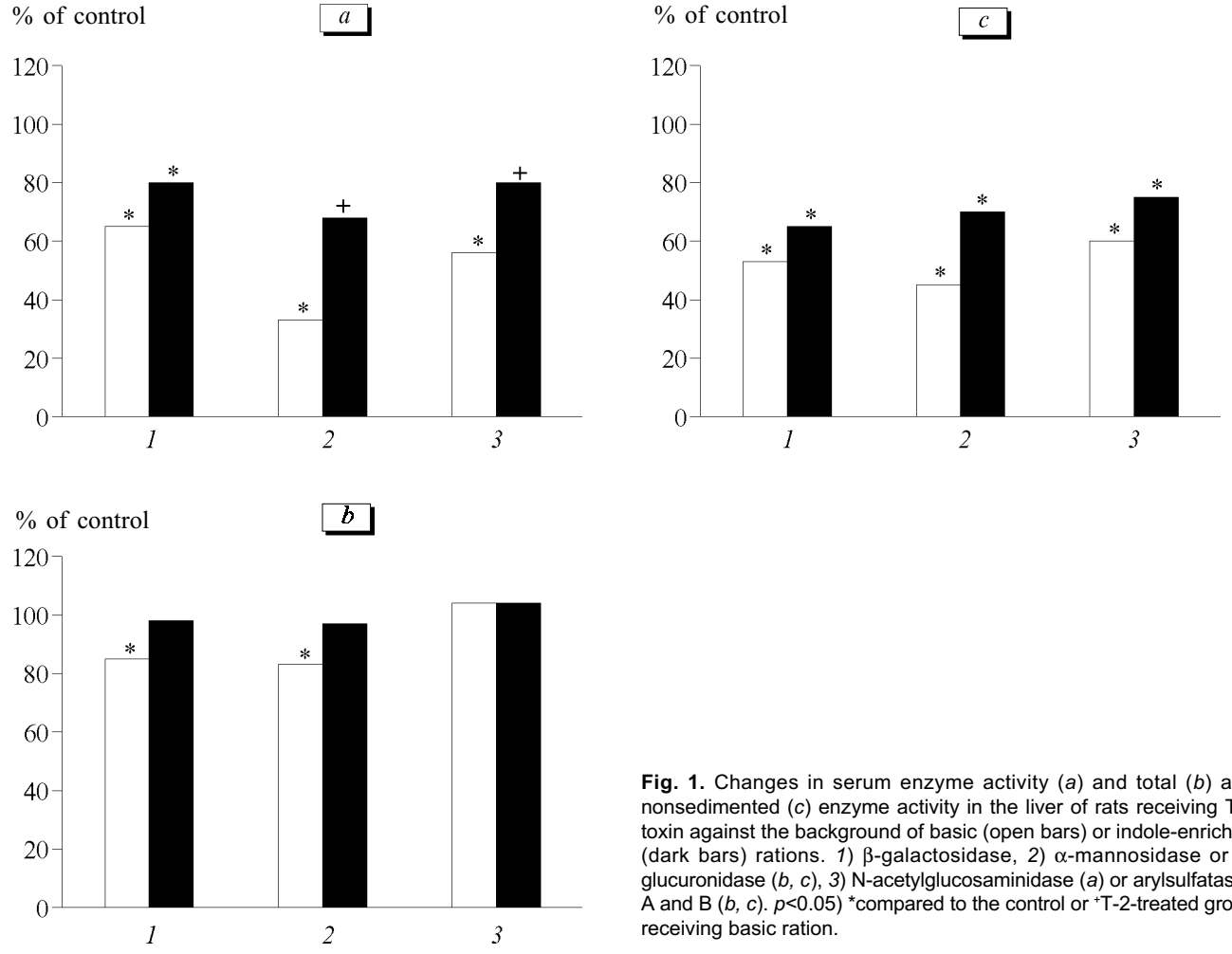

Fig. 1. Changes in serum enzyme activity (*a*) and total (*b*) and nonsedimented (*c*) enzyme activity in the liver of rats receiving T-2 toxin against the background of basic (open bars) or indole-enriched (dark bars) rations. *1*) β-galactosidase, *2*) α-mannosidase or β-glucuronidase (*b, c*), *3*) N-acetylglucosaminidase (*a*) or arylsulfatases A and B (*b, c*). $p<0.05$) *compared to the control or $^{+}$T-2-treated group receiving basic ration.

Cytoplasm vacuolation in smooth muscle cells of intraorgan arteries was observed in most rats of this group.

Adding food indoles to the rat ration was accompanied by significant attenuation of all manifestations of T-2 toxicity. Body weight of these rats did not differ from the control and increase in relative liver weight was less pronounced (by 11%). Macroscopic changes in the liver were revealed only in 50% rats. Histological and histochemical examinations of internal organs revealed lower changes in the liver, thymus, and intraorgan vessels in toxin-treated rats receiving indole-rich ration. In some animals these changes were absent. Inhibition of serum enzyme activity and nonsedimented activity of lysosome enzymes was also attenuated by indoles (Fig. 1). Activity of carboxylesterase and benzo(a)pyrene hydroxylase, cytochrome P-450 and microsomal protein contents did not differ from control values or significantly surpassed them (Fig. 2). In the whole, activity of xenobiotic metabolism enzymes in the liver of rats receiving indole-enriched ration was 1.5-2.0-fold higher than in toxin-treated rats receiving basic ration.

Thus, cruciferae indoles applied in the concentration, which has no unfavorable effect on experimental animals, modulate activity of xenobiotic metabolism enzymes in the liver and intestinal mucosa and attenuate T-2 toxicity.

The ability of cruciferae indoles and isothiocyanates, as well as cruciferae vegetables added to the ration, to modulate the activity of enzymes of xenobiotic metabolism was shown in experiments on rats [15]. It was shown that indole-3-carbinole and ascorbigen possess the properties of inductors of the dioxane type activating CYP1A1 and CYP1A2 and phenobarbital type inducing CYP2B and CYP3A [7]. It was shown that indolo[3,2-b]carbasole formed in the acid gastric medium from indole-3-carbinole and ascorbigen possesses higher affinity to Ah-receptor, an initial component activating transcription of genes of the cytochrome CYP1A1 family, UDP-glucuronosyl transferase 1A subfamily, including 1A6 (specific acceptor p-NP), and some isoforms of glutathione transferase and epoxydhydrolase [4,14]. Sulforafan present in high amounts in broccoli is a potent natural monofunctional inductor of phase II xenobiotic metabolism

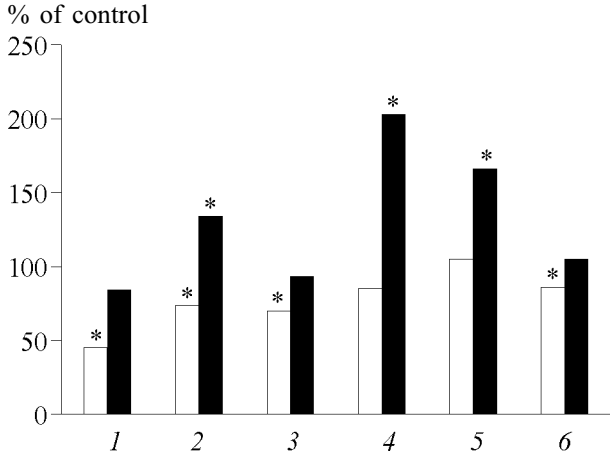

Fig. 2. Changes in activity of xenobiotic metabolism enzymes in the liver of rats receiving T-2 toxin against the background of basic (open bars) or indole-enriched (dark bars) rations. *1*) cytochrome P-450, *2*) benzo(a)pyrene hydroxylase, *3*) carboxylesterase, *4*) p-NP-UDP-glucuronosyl transferase, *5*) glutathione transferase, *6*) microsomal protein. **p*<0.05 compared to the control.

enzymes, glutathione transferase and NAD(P)H-quinone reductase [16].

The observed effect of nutritional indoles on T-2 toxicity probably depends on their ability to activate enzymes participating in biotransformation and detoxification of T-2 toxin. One of the main ways of T-2 detoxification both in the liver and small intestine is deacetylation with the formation of less toxic metabolites HT2-toxin, neosolaniol, T-2 tetraol, T-2 triol, involving microsomal carboxylesterase. Monooxygenases participate in the formation of hydroxyl derivatives of T-2 and HT-2 toxins, while greater part (about 70%) of metabolites is excreted as glucuronide conjugates. This assumption is confirmed by high functional activity of enzyme system participating in xenobiotic metabolism in the liver of toxin-treated rats receiving indole-enriched ration.

The analysis of our results and published data allow to conclude that food indoles can modulate the activity and activity balance of various enzymes including different isoforms of cytochrome P-450, epoxide hydrolase, glutathione transferases, sulfotranspherases, quinone reductases involved in xenobiotic and endobiotic metabolism, and change their final biological effects.

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