# XENOBIOTIC METABOLIZING ENZYMES AND COMPARATIVE TOXICITY OF TRICHOTHECENE MYCOTOXINS

L. V. Kravchenko, A. B. Levitskaya, and A. É. Kranauskas

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The trichothecene mycotoxins, whose toxic properties are associated with the presence of an epoxide in their structure, are metabolites of the microscopic molds of the *Fusarium* genus and they are particularly dangerous contaminants of food and forage products [5, 13]. A potential danger for human health may also arise from residual quantities of toxic metabolites of these mycotoxins in products of animal origin. The writers showed previously that the toxic action of the principal member of the trichothecenes, T-2-toxin, is characterized by considerable disturbances of the enzyme status of the liver, by a lasting decrease in the activity of several serum enzymes, and by leuko- and lymphocytopenia [2, 4, 12].

Some biochemical and hematologic parameters in mice with mycotoxicoses caused by HT-2 toxin, a deacetylated derivative of T-2-toxin formed in the liver of animals of different species with the participation of microsomal carboxylesterase (CE) [14, 15], were studied in the investigation described below.

## EXPERIMENTAL METHOD

Experiments were carried out on male CBA × C57BL/6 mice weighing 24-25 g. To determine $LD_{50}$ for HT-2-toxin, a solution of the toxin in 1% ethanol was injected once into the animals' stomach in doses of 10, 12, 14, 16, 18, and 20 mg/kg body weight. To study the toxic action of HT-2-toxin, it was injected into mice in a single dose of $LD_{50}$, or daily for 7 days in a dose of 1/5 $LD_{50}$, or for 14 days in doses of 1/10 and 1/50 $LD_{50}$. Equivalent volumes of solvent were injected into control animals. Blood was taken from the caudal vein of the animals 24 h before decapitation for determination of the hemoglobin concentration, total erythrocyte and leukocyte counts, and the leukocyte formula. The mice were decapitated 24 h after the last injection. The concentration of cytochrome P-450 [9], activity of CE [10] and of UDP-glucuronosyltransferase (UDP-GlT) [1], and the protein concentration were determined in liver homogenates prepared under standard conditions, and glutathione transferase (GT) activity was determined [8] in the cytosol (105,000 g, 60 min). Activity of alkaline phosphatase (AlP) [7] and lysozyme [11] was determined in the blood serum.

## EXPERIMENTAL RESULTS

A single injection of HT-2-toxin in a dose of more than 16 mg/kg caused the development of a picture of poisoning which was indistinguishable from acute T-2-toxicosis in mice [6]. The first signs of the toxic action of HT-2-toxin appeared 10 h after injection and reached maximal severity by the end of the first day. $LD_{50}$ for HT-2-toxin calculated by Kärber's method over a period of 72 h was 12.75 mg/kg, which was almost twice the value of $LD_{50}$ found for T-2-toxin for mice [6].

After a single injection of toxin in a dose equal to $LD_{50}$, or after daily injections in a dose of 1/5 $LD_{50}$ for 7 days, only adynamia and a small but significant decrease in body weight were observed in the animals. The mortality in both groups was 10%. The relative weight of the liver also was moderately (by 8 and 12%) increased in the mice, but the relative weight of the thymus was sharply reduced (by 48 and 62%). No signs of poisoning were ob-

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TABLE 1. Effect of HT-2-Toxin on Serum Enzyme Activity and Some Hematologic Parameters in Mice (M ± m, n = 7)

| Parameter | Experimental conditions | Dose of toxin, mg/kg | | | |
|---|---|---|---|---|---|
| | | 12.75 ($LD_{50}$) | 2.55 ($^1/_5$ $LD_{50}$) | 1.27 ($^1/_{10}$ $LD_{50}$) | 0.25 ($^1/_{50}$ $LD_{50}$) |
| AlP, μ moles/min/ml | Control | 0,181±0,015 | 0,182±0,028 | 0,200±0,016 | 0,200±0,016 |
| | Experiment | 0,148±0,016 | 0,112±0,007* | 0,183±0,020 | 0,202±0,010 |
| Lysozyme, μ g/ml | Control | 6,6±0,3 | 6,2±0,6 | 7,8±1,1 | 7,8±1,1 |
| | Experiment | 5,7±0,4 | 5,5±0,5 | 7,0±1,5 | 8,0±0,4 |
| Hemoglobin, g/liter | Control | | 154,7±3,5 | 174,0±4,9 | 174,0±4,9 |
| | Experiment | | 154,0±4,2 | 170,4±4,6 | 174,7±4,9 |
| Erythrocytes, $(\times 10^{12})$/liter | Control | | 6,3±0,1 | 7,1±0,3 | 7,1±0,3 |
| | Experiment | | 5,8±0,1* | 6,8±0,3 | 6,8±0,4 |
| Leukocytes $(\times 10^9)$/liter | Control | | 14,6±0,8 | 15,9±0,9 | 15,9±0,9 |
| | Experiment | | 10,7±0,9* | 11,2±1,2* | 15,7±0,7 |
| Neutrophils, percent | Control | | 20,2±3,5 | 21,8±2,3 | 21,8±2,3 |
| | Experiment | | 33,7±3,7* | 18,0±1,6 | 24,3±0,3 |
| Lymphocytes, percent | Control | | 74,7±3,3 | 71,2±5,3 | 71,2±5,3 |
| | Experiment | | 63,0±3,5* | 73,8±1,4 | 70,7±3,5 |

Legend. *P < 0.05.

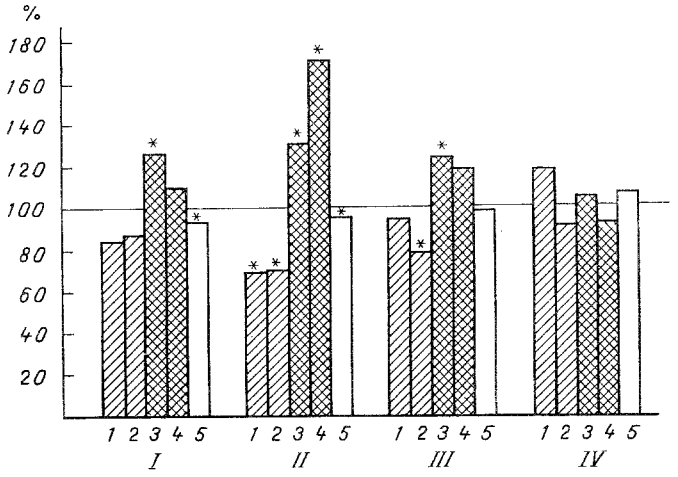


Fig. 1. Changes (in percent) in activity of xenobiotic metabolizing enzymes and protein concentration in liver of mice receiving HT-2-toxin in a single dose of $LD_{50}$ (I), a dose of 1/5 $LD_{50}$ daily for 7 days (II), and a dose of 1/10 $LD_{50}$ (III) or 1/50 $LD_{50}$ (IV) daily for 14 days. 1) Cytochrome P-450; 2) CE; 3) UDP-GlT; 4) GT; 5) protein. *P < 0.05.

served in mice receiving 1/10 or 1/50 $LD_{50}$ of the toxin. On the whole, when equal doses (calculated as $LD_{50}$) were used the severity of the symptoms of HT-2-toxicosis was less than in T-2-toxicosis.

It was shown previously that injection of T-2-toxin into mice in doses exceeding 1/50 $LD_{50}$ depresses AlP (by 30-50%) and lysozyme (by 15-75%) activity in the blood serum and causes a decline (by 20-50%) in the number of leukocytes and lymphocytes in the blood [6]. As Table 1 shows, AlP activity and the lysozyme concentration in the blood serum were moderately reduced only in animals receiving $LD_{50}$ or 1/5 $LD_{50}$ of HT-2-toxin. In mice receiving 1/5 $LD_{50}$ of HT-2-toxin, moderate leuko- and lymphocytopenia and neutrophilia were found. The leukocyte count also was reduced in animals receiving 1/10 $LD_{50}$ of the toxin daily for 14 days. When the toxic action of HT-2-toxin is assessed on the basis of hematologic parameters and the degree of changes in blood enzyme activity, it can thus also be concluded that its toxicity for mice is less than that of T-2-toxin.

Data on changes in activity of xenobiotic metabolizing enzymes in HT-2-toxicosis are interesting (Fig. 1). In the liver of mice receiving the toxin in the maximal dose, for instance, a moderate fall was observed in concentrations of cytochrome P-450 and CE, whereas activity of the key enzymes of phase II of metabolism increased. These changes were much more marked in the animals receiving 1/5 $LD_{50}$ of the toxin: The cytochrome P-450 level and CE activity

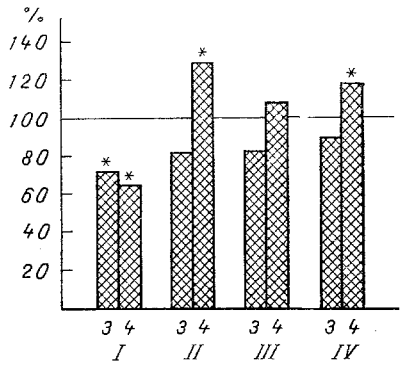


Fig. 2. Changes (in percent) in UDP-G1T (3) and GT (4) activity in liver of mice receiving T-2-toxin in a single dose of $LD_{50}$ (I), in a dose of 1/50 $LD_{50}$ daily for 7 days (II), and a dose of 1/10 $LD_{50}$ (III) or 1/50 $LD_{50}$ (IV) daily for 14 days.

fell to 70% of the control values, whereas UDP-G1T and GT activity rose to 130 and 170%, respectively. In animals receiving the same total dose of HT-2-toxin, but spread over a period of 14 days (1/10 $LD_{50}$ per dose), the changes in enzyme activity were in the same direction as before, but they were less marked. In a dose of 1/150 $LD_{50}$ the toxin had no significant effect on enzyme activity.

Previously, in a study of chronic T-2-toxicosis in mice, the writers established dose-dependent depression of activity of enzymes responsible for phase I of xenobiotic metabolism, with at the same time an increase in GT activity [4]. These results, and those of a study of the effect of various modifiers of activity of xenobiotic metabolizing enzymes, led the writers to postulate that one of the main pathways for detoxication of trichothecenes in the body is through conjugation reactions [3]. Data on changes in UDP-G1T and GT activity in the liver of CBA × C57BL/6 mice in response to injection of T-2-toxin in the same doses, and by the same scheme, as were used for injection of HT-2-toxin, are given in Fig. 2. By contrast with HT-2-toxin, T-2-toxin injected in a single dose of $LD_{50}$ caused marked depression of enzyme activity. UDP-G1T activity remained below the control level after administration of all doses tested, but GT activity rose moderately if T-2-toxin was injected in doses of under $LD_{50}$.

HT-2-toxin is thus less toxic for mice than T-2-toxin (higher value of $LD_{50}$, less marked clinical and hematological manifestations of poisoning, and their later appearance). The ability of HT-2-toxin to activate conjugation reaction by a greater degree than T-2-toxin is perhaps one factor determining the lower toxicity of HT-2-toxin.

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# RELATIONS BETWEEN NATURAL ANTIOXIDANT CONTENT AND VISCOSITY OF LIPIDS IN NORMAL ORGANELLE MEMBRANES

E. B. Burlakova, A. N. Goloshchapov, and R. F. Kerimov

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Strict correlation between structure and function is a characteristic feature of biological membranes, which are cooperative systems [7]. An important role in the function of membranes is known to be played by lipid–protein interactions, a significant effect of which is exerted by processes of lipid peroxidation (LPO) [12]. Changes in the LPO level in membranes lead to changes or even disturbances of their functional activity [11]. It has been shown that intensification of LPO increases the viscosity of membrane lipids [12]. The "flowability" of the lipid bilayer in turn affects activity of systems generating free radicals in lipids. For instance, it was shown in [8] that LPO is initiated only under conditions permitting high flowability of membrane lipids, whereas an increase in viscosity of the lipids causes slowing of LPO [2].

The system of natural antioxidants (NAO), found in lipids, is one of the systems that controls the LPO level in membranes. NAO inhibit excessive LPO through exchange reactions, and they thereby maintain the structural integrity of the membranes and its functional activity. The view is held that antioxidants, built into the lipid bilayer of the membrane, may also have a direct effect on the structure of the membrane [10], and may thus modify accessibility of the membrane lipids to oxygen.

The aim of this investigation was accordingly to establish relations between the content and "efficiency" of NAO, on the one hand, and the viscosity of membrane lipids of subcellular fractions differing in the velocity of LPO reactions, on the other hand. For this purpose the content and "efficiency" of NAO and the viscosity of the lipid bilayer of the membranes in different organelles of liver cells of intact mice were determined.

## EXPERIMENTAL METHOD

Organelles (nuclei, mitochondria, and microsomes) were isolated by differential centrifugation [4, 5, 9] from the liver of noninbred mice weighing 18-22 g. Lipids were extracted from these organelles by Folch's method in Kates' modification [6].

The antiradical activity (ARA) of the lipids and the NAO level were determined on a chemiluminescence model of initiated oxidation of ethylbenzene [1].

The viscosity of the membrane lipids, and also thermoinduced structural transitions taking place in them, were determined by the spin probe method [3]. A stable iminoxyl radical — [2,2,6,6]-tetramethyl-4-caprylolyoxypiperidiene-1-oxyl — was used as the probe. The EPR spectra were recorded on an E-4 EPR spectrometer (Varian, USA).

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