Bull. Environ. Contam. Toxicol. (2000) 64:586-592

DOI: 10.1007/s001280000043

Bulletin of Environmental Contamination and Toxicology

Liver Enzyme Activities of Rats Exposed to Ochratoxin A and T-2 Toxin with Antioxidants

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Received: 7 July 1999/Accepted: 26 January 2000

Ochratoxin A (OTA) is a mycotoxin produced by moulds from the Aspergillus and Penicillium genera. It is a natural contaminant of a wide variety of both human and animal foodstuffs. Biochemical changes that occur following treatment with OTA involve almost all organs and tissues. Ochratoxin A causes glucosuria, a decrease in the transport of organic anions, and mitochondrial dysfunction in the kidney (Gekle and Silbernagl 1994). Inhibition of mitochondrial respiration and oxidative phosphorylation, as well as decreased rates of protein and RNA synthesis, were described in liver (Dirheimer and Creppy 1991).

T-2 toxin is a potent member of the family of trichothecene mycotoxins produced by Fusarium fungi. Both humans and animals are susceptible to intoxication from food contaminated by T-2 toxin. T-2 toxin has been shown to induce alimentary intoxication, lymphatic necrosis or atrophy and, among others, haematological symptoms such as anemia and leukopenia. It has been reported that T-2 toxin is a potent inhibitor of protein and DNA synthesis in mammalian cells (Atroshi et al. 1995).

Aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) have long been used as sensitive indicators of liver diseases in humans and have been regarded as being virtually liver specific (Wills 1985). Increases in serum activities of ALAT, considered a liver-specific enzyme in rats, are used as markers of hepatocellular necrosis or increased cell membrane permeability (Clampitt and Hart 1978; Boyd 1983). Changes in the chemical variables occurred frequently, indicating that clinical chemical evaluations can be useful for detecting the onset and progression of treatment effects or transient effects.

The involvement of certain micronutrients in the antioxidant defense system against free radicals of reactive oxygen molecules has been reported. Under normal conditions, potentially toxic oxygen-free radicals are primarily generated by mitochondrial respiratory metabolism and are efficiently neutralized by cellular antioxidant defenses (Halliwell 1992). Thus, the antioxidants present in food are particularly appropriate for minimizing the toxic and carcinogenic effects of some mycotoxins (Rizzo et al. 1994).

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The presence of mycotoxins in the environment increases the risk factor for animals and human. The potential for immune system exposure to immunotoxins in the environment is well documented. Antioxidants have been shown to counteract the production of lipid peroxides and the corresponding toxic signs of myctoxins. Therefore, a study was conducted to assess the fluctuations in rat liver enzymes induced by T-2 toxin and OTA and further to assess the protective roles of the antioxidants tamoxifen, vitamin E, and selenium against toxicity caused by mycotoxins.

MATERIALS AND METHODS

A total of 84 male rats approximately 4 wk of age and weighing 200-220 g were obtained from the National Veterinary and Food Research Institute stabularium. The animals were randomly divided into 7 treatment groups of 12 animals per group. For 2 wk prior to the start of the study the animals were exposed to a reverse light/dark rhythm (Bursch et al. 1985). During this period and throughout the subsequent test period the rats were given water ad libitum with standard pelleted diet made available for 5 hr each day between 09.00 and 14.00 hr. The animals were grouped as follows: the first group of animals received 0.5 mL of seed oil orally and served as controls; the second and third groups received OTA or T-2 toxin only; the fourth and fifth groups received the antioxidants (vitamin E + Se or Tamoxifen); the sixth and seventh groups received OTA or T- 2 toxin and antioxidants.

The animals, except the control group, were fed for 4 wk with a constant semi-synthetic feed (Drepper and Weik 1972), deficient in vitamin E + selenium (ANALYCEN, LIDKÖPING, SWEDEN). The feed contained 10% of a mixture of soya oil and lard (1: 1), 2% cellulose powder, 26 % maize starch, 20% casein, and 6% of a mixture of mineral salts. The rats in the antioxidant supplementation group were given a dose of 30 IU vitamin E/kg^{-1} diet (dl-α-tocopherol), 1 mg selenium/kg^{-1} diet (sodium selenite) or 1.5 mg tamoxifen/ kg^{-1} diet (tamoxifen citrate) for 8 wk. Ochratoxin A and T-2 toxin were dissolved in seed oil and administered orally at a dose level of 2.5 mg/kg and 2.8 mg/kg body weight respectively by gastric intubation. Control animals received seed oil only. The aim was primarily to produce acute toxicity. The animals were decapitated 24 hr after toxin administration. The liver was removed and rinsed with ice-cold homogenising buffer (50 m*M* Tris, 0.1 m*M* EDTA, pH 7.6). The supernatant and the rest of the tissue samples were frozen in liquid N_2 and stored at -85 “C.

Tissue enzyme activities of L-aspartate 2-oxoglutarate aminotranferase, ASAT, EC 2.6.1.1; L-alanine 2 oxoglutarate aminotransferase, ALAT, EC 2.6.1.2; L-γ – glutamyltransferase, γ-GT, EC 2.3.2.2.; and alkaline phosphatase, AP, EC 3.1.3.1 were determined after centrifugation of the samples (12,000xg in Eppendorf). Ten µL NONIDET-P-40 (octylphenoxy)-polyethoxyethanol), was then added to the supernatant, mixed and enzyme activities were determined using a Gilford System 3500 Computer Directed analyzer (Gilford Instrument Company, Oberlin,OH) according to the recommendations of the Committee on Enzymes of the Scandinavian

Society for Clinical Chemistry and Clinical Physiology (1974).

The data were analyzed by analysis of variance, and where statistically significant differences were found, they were then evaluated with a Student's t-test. The comparisons made (Atroshi et a1.,1995) were: control versus antioxidant-supplemented, toxin-treated versus non toxin-treated, antioxidant-supplemented with toxin versus antioxidant-supplemented without toxin, toxin-treated versus toxin plus antioxidants treated.

RESULTS AND DISCUSSION

The present investigation focused on the biochemical aspects and mode of action of toxic effects caused by T-2 toxin and OTA. Liver tissue enzymes, ASAT, ALAT and AP as well as others, are commonly elevated following cellular damage as a result of enzyme leakage from cells to the blood (Bogin et al., 1994).

As shown in Figures 1 and 2, there were significant changes in most of the liver tissue enzymes studied. While ASAT, ALAT, AP, and γ-GT activities were significantly higher in T-2 treated rats than in control groups (Figure 1), the level of protein was significantly lower. The AP level in the livers of rats treated with T-2 toxin was 292% greater than control group. Aspartate aminotransferase and ALAT activities also showed increases of 30% and 18%, respectively when compared to animals not treated with T-2 toxin. However, the protein concentrations in rat livers treated with T-2 toxin showed a decrease of 24% compared to the control group. When antioxidant supplementation to rats treated with T-2 toxin was considered, AP activity showed a significant decrease (71%) with tamoxifen supplementation and a 40% decrease with vitamin E + selenium supplementation. γ-GT activity showed a 20% and 17% decrease in rats adiministered tamoxifen and vitamin E + selenium, respectively. However, protein concentration increased by 8% after vitamin E + selenium supplementation (Figure 1).

The activities of ASAT, ALAT, AP, and γ-GT, and protein concentration, in rat liver treated with OTA are shown in Figures 2. A similar pattern of increases in liver enzyme activities to that in T-2 toxin treated rats was seen in OTA treated animals. Alkaline phosphatase levels showed a 317% increase compared to control animals, whereas ASAT and ALAT showed 41% and 25% increases, respectively. However, protein concentration decreased by 20% in rats treated with OTA. When antioxidant supplementation effect was evaluated, animals supplemented with antioxidants and then treated with OTA showed a decrease in liver enzyme activities compared to the control animals (Figure 2).

The kidney and liver are proposed to be the major target organs for environmental contaminants such as OTA (Marquardt and Frohlich 1992). Elevated liver enzymes are a common clinical problem of varying significance. ASAT and ALAT activities have long been used as sensitive indicators of liver diseases in humans and have been

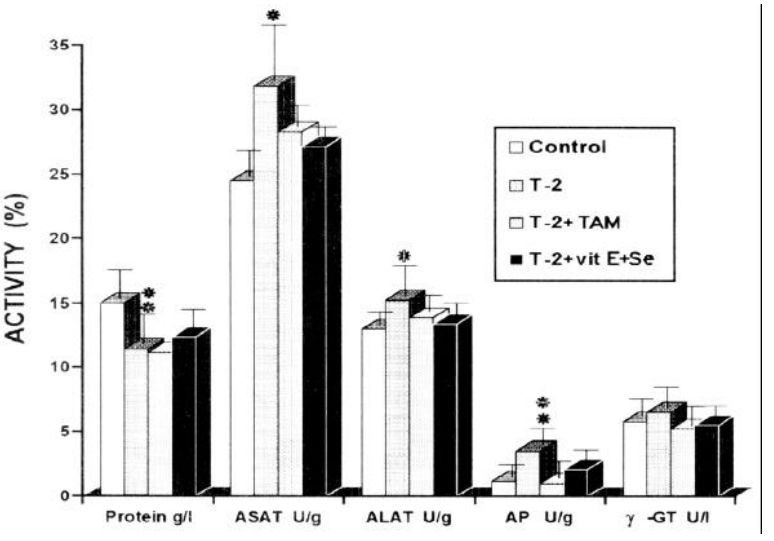

Figure 1. Enzymatic activity of liver tissue and protein concentration in rats treated with T-2 toxin, tamoxifen, or vitamin E + selenium. Results are mean ± SEM for 10 rats per group. The asterisk (*) indicates that the toxin group is significantly different from the control and the toxin plus antioxidants group. The activity percentage (mean&SE) is measured by comparing the enzymatic activity from its zero-time control (*P<0.05; **P<0.01).

regarded as being virtually liver specific (Wills, 1985). Although these enzymes have a less specific nature in the rat (Davy et al. 1988), higher plasma activities have been found in response to oxidative stress (Shimuzu et al. 1989). Exposure of experimental animals and humans to the trichothecenes, T-2 toxin and deoxynivalenol (Rizzo et al. 1994; Atroshi et al. 1994), and to OTA (Zanic-Grubisic et al. 1995), has been associated with altered parameters of humoral-mediated immunity and of the activities of various tissue enzymes.

Addition of vitamin E + selenium decreased the toxic effects caused by T-2 toxin. However, the level of protein was increased, which may suggest that some of the toxic effects caused by the toxins may be reduced by using vitamin E + selenium.

The significant changes in the liver activities of ASAT, ALAT, AP, and γ-GT after T-2 toxin and OTA administration indicate an association with biochemical alterations that could change when tamoxifen or vitamin E + selenium are adequately supplied.

Free radical production in cells may be greatly increased by certain toxic foreign compounds (Cheesman and Slater 1993). Mycotoxins as an example of toxic compounds also exert their toxicity via the production of free radicals and lipid peroxidation. This leads to the conclusion that the central mechanism of toxicity may be oxidative stress. Lipid peroxidation is a chain reaction resulting from the spread of highly reactive polyunsaturated fatty acid radicals, initiated by the attack of hydroxy

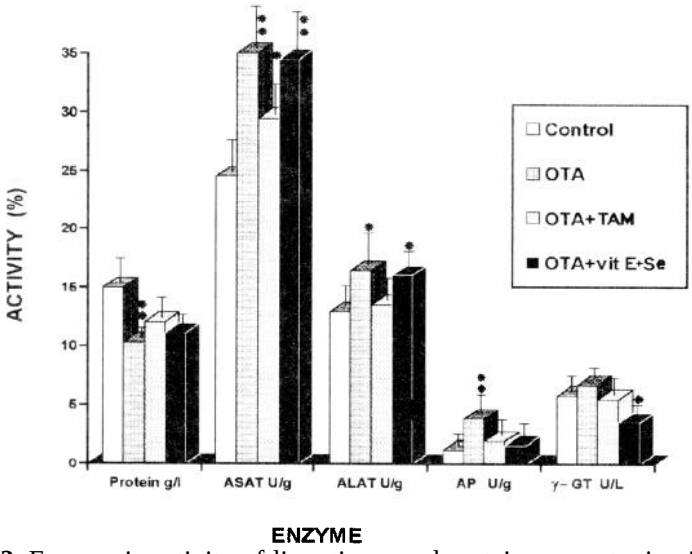

Figure 2. Enzymatic activity of liver tissue and protein concentration in rats treated with ochratoxin A tamoxifen, or vitamin E + selenium. The asterisk (*) indicates that the toxin group is significantly different horn the control and the toxin plus antioxidants group. The activity percentage (mean± SE) is measured by comparing the enzymatic activity horn its zero-time control (*P<0.05; **P<0.01).

radicals on the unsaturated bonds of membrane phospholipids (Rizzo et al. 1992; 1994). The liver has an elaborate antioxidant defense system and is relatively well protected against so-called “oxidative stress”. However, the generation of reactive free radicals induced by toxicants can overwhelm the antioxidant defenses in the liver and may result in tissue damage. Therefore, it is possible to postulate the involvement of a free radical mechanism in the toxicity caused by mycotoxins. In many cases, the production of free radicals may be secondary to the initial toxic mechanism, a consequence rather than the direct cause of cell damage. Chemopreventive antioxidant activities include scavenging reactive electrophiles (GSH-enhancing agents), scavenging oxygen radicals (vitamin E), and inhibiting arachidonic acid metabolism (tamoxifen) (Kelloff et al. 1994).

Administration of tamoxifen together with T-2 toxin or OTA significantly reversed the effects caused by the toxins alone, supporting the hypothesis that T-2 toxin and OTA toxicity may be reduced by using this antioxidant. Tamoxifen (TAM) is the antiestrogen most widely used in the chemotherapy and chemoprevention of breast cancer (Wiseman 1994). It has been reported that TAM and its more active metabolite 4-hydroxytamoxifen (OHTAM) induce multiple cellular effects, including antioxidant actions (Custodio et al. 1994). However, these drugs are not typical chain-breaking antioxidant compounds as compared with vitamin E. Tamoxifen has been shown to reverse hepatic toxicity caused by adjuvant chemotherapy in breast cancer patients. Among the liver enzymes measured, alkaline phosphatase, aspartate acetyl transferase, and gamma glutamyl transferase levels were reduced significantly in patients receiving

tamoxifen (Hirvikoski et al.1997). These data show that oxidant damage in liver tissues caused by OTA and T-2 toxin can be significantly reduced by pretreatment with antioxidants. These *in vivo* findings support the role of oxidant stress in the pathogenesis of liver toxicity.

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