

In Vitro Metabolism of T-2 Toxin

ROBERT A. ELLISON AND FRANK N. KOTSONIS

School of Pharmacy, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 11 October 1973

Incubation of T-2 toxin with the $9,000 \times g$ supernatant fluid of both human and bovine liver homogenate resulted in conversion to a single, deacetylated product identified as HT-2 toxin. Metabolism is more rapid in human liver. HT-2 toxin was not produced when human plasma was the incubating medium nor was it produced by treatment of T-2 toxin with simulated gastric juice. T-2 toxin was stable in gastric juice for at least 1 h.

The genus *Fusarium* is a widely occurring fungus which has caused substantial economic loss due to its pathogenicity towards crops used as feeds and foods (3). Associated with *Fusarium* infection of cereal grains have been several characteristic mycotoxicoses in both animals and humans resulting from ingestion of toxic metabolites produced by various strains of the fungus (5, 9). One of these, T-2 toxin (3-hydroxy-4,15-diacetoxy-8-[3-methylbutyryloxy]-12,13-epoxy- Δ^9 -trichothecene) (Fig. 1), produces radiomimetic lesions of the liver, gastrointestinal tract, and skin and may be at least partly responsible for some of the symptoms of moldy corn toxicosis experience in the midwestern United States.

Recently we demonstrated that, although lethal at higher doses (pigeon oral mean lethal dose = 2.75 mg/kg), T-2 toxin is an efficient emetic at lower doses (pigeon oral mean effective dose = 0.72 mg/kg) (4). Emesis in both animals and humans has been reported as a distinct symptom associated with infection of grains by some *Fusarium* strains (6). We felt it of value to determine whether the biological activity could be ascribed to T-2 toxin or rather to a metabolite.

Because trichothecenes can be rearranged and/or hydrolyzed in the presence of acid or base (2, 10), it was necessary to determine first whether T-2 toxin would survive conditions in the gastrointestinal tract. When T-2 toxin was exposed to simulated gastric juice (7) for 1.2 h at 37 C, only a trace amount of a new compound could be detected on thin-layer chromatography (TLC). After 24 h at 25 C, three new spots at lower R_f appeared in addition to residual T-2 toxin. These were not investigated but were presumed to be hydrolysis products. It appears, then, that the toxin is absorbed into the blood stream intact.

Human liver, stored at 0 C for 14 months, was obtained via university hospitals from a 22-year-old male who died of pulmonary edema. Both human and bovine $9,000 \times g$ supernatants were prepared as follows and incubated identically with the concentrations indicated. Liver at 4 C was washed with isotonic potassium chloride solution buffered to pH 7.5 with 0.05 M tris(hydroxymethyl)aminomethane buffer. This was minced and homogenized in a similar solution for 3 min at low speed in a Waring blender followed by 5 min in a Potter-Elvehjem hand homogenizer. The resulting homogenate was centrifuged at 10,000 rpm at 0 C for 20 min, and the supernatant fluid was used for incubation. The incubating solution (47 ml) consisted of the following final concentrations: liver, 85 mg equiv/ml; NADP, 0.94×10^{-4} M; glucose-6-phosphate, 2.13×10^{-3} M and T-2 toxin, 0.83×10^{-5} M (18.3 mg). The incubation proceeded for 75 min at 37 C with periodic TLC analysis. Upon completion, the solution was extracted three times with chloroform, and the extracts were dried over sodium sulfate and evaporated to give a residue (36 mg) which was column chromatographed in 1-ml fractions with ethylacetate-Skelly B (5.7:1) as eluent. Fractions 120 to 190 contained a pure compound (14 mg) which was compared with HT-2 toxin by mass spectrometry, pmr spectrometry, and mobility in four TLC solvent systems: ethyl acetate, $R_f = 0.25$; toluene-ethyl acetate (1:3), $R_f = 0.12$; ethanol-ethylacetate-acetone (4:4:1), $R_f = 0.66$; ethanol-ethyl acetate-acetone (1:4:4), $R_f = 0.64$.

Incubation of T-2 toxin with the $9,000 \times g$ supernatant fluid of human liver resulted in the appearance on TLC of a new, more polar product after 10 min. Isolation and characterization of this compound by proton magnetic resonance and mass spectrometry as well as

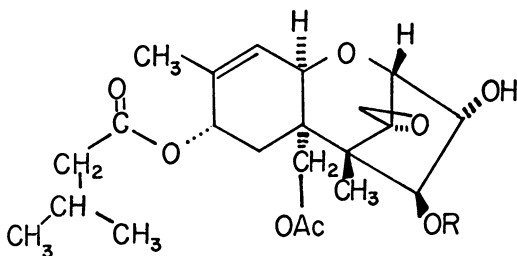


FIG. 1. Chemical structure of T-2 toxin and HT-2 toxin. R = Ac, T-2 toxin; R = H, HT-2 toxin.

TLC comparison in four solvent systems showed it to be identical with HT-2 toxin (reference 1; see Fig. 1). By TLC analysis, the half-life of T-2 toxin in this system was found to be 20 ± 5 min. This metabolite could not be produced by incubation with cofactors alone or by incubation with cofactors in the presence of blood expressed from the liver. Thus, production of HT-2 is most likely due to a liver esterase. This enzyme cleaves the same acetyl group which can be selectively removed by treatment of T-2 toxin with aqueous ammonia (10). Excess material obtained during the extraction procedure did not interfere with TLC analysis, and HT-2 toxin could be recovered in 84% yield after chromatography. Under the same conditions, bovine liver also produced HT-2 toxin but more slowly. The half-life of T-2 toxin in this system was found to be 40 ± 5 min.

Intravenous injection of solutions of HT-2 toxin in 27% ethanol-physiological saline into three pigeons at doses of 0.68, 0.74, and 1.32 mg/kg resulted in prompt vomiting. Due to the relatively short induction time for emesis induced by T-2 toxin (4), it is impossible to conclude whether both toxins or only HT-2 toxin is responsible for emesis. Both compounds have, however, been shown to be equipotent in the rabbit reticulocyte bioassay and differ only by a factor of 1.7 in toxicity toward mice (8). In this study, the pigeon given the highest dose died between 30 and 40 h after administration, which also suggested that the two compounds are of comparable toxicity.

Although in vivo metabolism studies must still be made, it appears that the deacylation reaction does not dramatically reduce the toxicity of ingested T-2 toxin. Further, since HT-2 production by *F. tricinctum* is apparently not restricted to a low-temperature requirement as opposed to T-2 production (1), the potential for emetic activity may be greater than originally supposed.

We thank C. J. Sih for kindly supplying the human liver and Oscar Mayer and Co. for the bovine liver. We are grateful to H. L. Burmeister, USDA Northern Regional Research Laboratories, Peoria, Ill., and Ru-dong Wei, Department of Biochemistry, University of Wisconsin, Madison, for samples of T-2 toxin and HT-2 toxin, respectively. We thank H. K. Schnoes, Department of Biochemistry, University of Wisconsin, Madison, for some of the mass spectra.

This work was supported by funds from the Graduate School, University of Wisconsin.

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