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Note

Antiviral Activity of Metabolites of T-2 Toxin against Herpes Simplex Virus Type 2

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T-2 toxin (T-2), 4β,15-diacetoxy-8α-(3-methylbutyryloxy)-3α-hydroxy-12,13-epoxytrichothecec-9-ene, is a trichothecene mycotoxin produced by various species of *Fusarium*, and is one of the most important trichothecene mycotoxins occurring naturally in various agricultural products.[1,2] T-2 is possibly involved in serious field cases of human toxicoses and farm animals, and is a potent inhibitor of protein and DNA synthesis in cultured mammalian cells.[1] T-2, when orally administered to rodents, chickens, and a lactating cow, is rapidly metabolized into various products by two major pathways including hydrolysis[3-6] and hydroxylation[7,8] (Fig. 1). The other pathway including deepoxidation and glucuronide conjugation has been also described.[9,10] Recently, we reported that T-2, diacetoxyscirpenol and neosolaniol (NEOS) inhibited herpes simplex virus type 2 (HSV-2) replication by blocking viral early protein synthesis but not adsorption and penetration of HSV-2 virions into the host cells and syntheses of viral DNA and RNA, suggesting that a viral plaque-reduction test was useful for analysis of the structure–activity relationship in terms of the inhibition of viral protein synthesis by trichothecenes.[11,12] Therefore, we evaluated the antiviral activity of T-2 metabolites against HSV-2. This paper reports that the metabolic conversion of T-2 to 3′-hydroxy HT-2 toxin (3′-OH-HT-2) or to T-2 tetraol (TOL) decreases the antiviral activity.

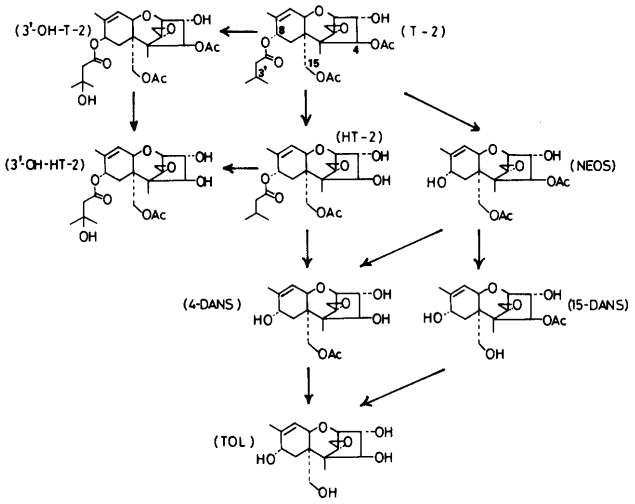

Fig. 1. Chemical Structures of Metabolites of T-2 Toxin.

T-2, T-2 toxin; HT-2, HT-2 toxin; 3′-OH-T-2, 3′-hydroxy T-2 toxin; 3′-OH-HT-2, 3′-hydroxy HT-2 toxin; NEOS, neosolaniol; DANS, deacetylneosolaniol; TOL, T-2 tetraol.

Table I. Antiviral Activity of T-2 Metabolites against HSV-2

Trichothecene	IC_{50}(ng/ml)	
T-2	2.5	
3′-OH-T-2	6.0	(2.4)
3′-OH-HT-2	105.0	(42.0)
HT-2	5.7	(2.3)
NEOS	65.0	(26.0)
4-DANS	100.0	(40.0)
15-DANS	85.0	(34.0)
TOL	195.0	(78.0)

Each value is the mean of duplicate assays. Values in parentheses represent the ratio of the IC_{50} for T-2 metabolites to that for T-2.

Strain 186 of HSV-2 was used in this study. Human epidermoid carcinoma No. 2 (HEp-2) cells as host cells for HSV-2 were grown in Eagle's minimum essential medium supplemented with 10% fetal calf serum. The chemical structures of T-2 and its metabolites used in this study are shown in Fig. 1. T-2, HT-2 toxin (HT-2), and NEOS were isolated from cultures of *Fusarium sporotrichioides*.[13] 3′-Hydroxy T-2 toxin (3′-OH-T-2) and 3′-OH-HT-2 were synthesized from T-2 as described previously.[7] TOL and 15-deacetylneosolaniol (15-DANS) were obtained by alkaline hydrolysis of T-2 and NEOS, respectively. 4-Deacetylneosolaniol (4-DANS) was prepared by incubating HT-2 with the S-9 fraction of mice liver.[8] The purity of these toxins was over 98%, confirmed by gas-liquid chromatography.[14] All toxins were dissolved in dimethyl sulfoxide at a concentration of 20 mg/ml. Stock solutions (200 μg/ml) were prepared, passed through a 450-nm membrane filter (Millipore Corp., Bedford, MA, U.S.A.), and stored at −20°C until use.

Because the toxins did not cause any cytotoxic alterations of the cells at 0.5 μg/ml, the antiviral activities of the trichothecenes were examined at doses less than this concentration by a plaque-reduction test as described previously.[15] The HSV-2 plaque formation was inhibited by the trichothecenes in a dose-dependent manner, and the concentrations required for the 50% inhibition of HSV-2 plaque formation (IC_{50}) are shown in Table I. As for the structure-antiviral activity relationship of T-2 metabolites, the hydroxylation at the C-3′ position of T-2 to 3′-OH-T-2 toxin and hydrolysis at the C-4 position of T-2 to HT-2 did not significantly decrease the antiviral activity. The hydroxylation at the C-3′ position of HT-2 to 3′-OH-HT-2, hydrolysis at the C-4 position of 3′-OH-T-2 to 3′-OH-HT-2, and hydrolysis at the C-8 position of T-2 to NEOS or HT-2 to 4-DANS caused more than 17-fold decrease in their antiviral activity. After hydrolysis at the C-8 position, the hydrolysis at the C-15 position of 4-DANS to TOL or both at the C-4 and C-15 positions of NEOS to TOL did not greatly affect the antiviral activity. These results indicate that both hydrolysis at the C-4 position and hydroxylation at the C-3′ position or hydrolysis at the C-8 position of T-2 are responsible for the decreased antiviral activity.

The acute toxicity of T-2 metabolites has been investigated in various animal species.[14,16] When mice were administered intraperitoneally with the T-2 metabolites, it was demonstrated that toxicity of T-2 was similar to that of 3′-OH-T-2 and HT-2 but 3- to 6-fold higher than that of the other metabolites.[14] Furthermore, T-2 is also metabolized into T-2 triol formed by hydrolysis at the C-15 position of HT-2.[17] Forsell *et al.*[18] showed that the metabolic conversion of T-2 to 3′-OH-HT-2 or to T-2 triol and TOL greatly decreased the cytotoxicity to lymphoid cells. Therefore, these and our studies support the idea that the biological activity of T-2 can be reduced in at least two ways; one way is two substitutions of hydroxy groups at either the C-4 and C-3′ or C-4 and C-15 positions and the other way is a single substitution of a hydroxy group at the C-8 position.

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