Note

Confirmation of DOM-1, a De-epoxidation Metabolite of Deoxynivalenol, in Biological Fluids of Lactating Cows

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Deoxynivalenol (DON), 3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one, is a mycotoxin produced by Fusarium graminearum,1) F. culmorum,2~4) F. solani var. coeruleum,5) F. saubucinum,5) F. avenaceum2) and Gerlachia nivalis,2) and is one of the most important trichothecene mycotoxins occurring naturally in several cereals and feed grains throughout the world. The toxin was also found together with nivalenol in commercial cereal foods.6) Moreover, since animal feed supplies may be contaminated, there is a possibility that animal products for human consumption may also be contaminated with residual DON and its metabolites. Yoshizawa et al.7) demonstrated that DON was metabolized in vivo in rodents into de-epoxidized DON (DOM-1), which was found in the plasma, liver and excreta. This report describes the confirmation of the presence of this metabolite in cows milk as well as other biological samples from cows.

Lactating Holstein cows were fed twice a day for 5 consecutive days with a ration spiked with naturally DON-contaminated corn (the DON level in the feed averaged 66±11 (S.E.) ppm). Milk and excreta samples were collected twice a day; selected blood samples were collected on day 5; and aliquots of individual samples were kept frozen until required.

Extraction and clean-up were carried out in accordance with the procedures previously described8) with some modifications. The milk samples (50ml) were homogenized with acetone (150ml) and then filtered by suction. The residue was rinsed with acetone (20ml×2). The combined filtrate was evaporated to about 30ml in vacuo, and the remaining aqueous solution was partitioned with n-hexane (50ml). The organic layer was back-extracted with acetonitrile (10ml). The acetonitrile layer was combined with the aqueous layer followed by concentration in vacuo to an about 20ml aqueous solution, which was introduced onto a preconditioned Amberlite XAD-4 column (1×10cm). The column was eluted sequentially with 30ml of water and then 40ml of methanol. The methanol eluate was concentrated and then introduced onto a column containing Florisil (5g, 1cm i.d.) packed in chloroform–methanol (9:1, v/v) and topped with a 1cm layer of anhydrous sodium sulfate. The column was eluted with 50ml of chloroform–methanol (9:1, v/v). After evaporation of the solvent, an aliquot of the residue was derivatized to trimethylsilyl (TMS) ethers by treatment with a mixture of trimethylsilylimidazole-bis-(tri-methylsilyl)acetamide-trimethylchlorosilane (3:3:2, v/v/v) followed by quantitation by gas chromatography with a 63Ni-electron capture detector (GC-ECD), gas chromatography-mass spectrometry (GC-MS) or GC-MS-mass fragmentography (MF).

A 40-ml portion of each urine sample was chromatographed successively on Amberlite XAD and Florisil columns as described above. Feces were extracted in accordance with the procedure previously described.8) Plasma samples (5 to 13ml) were diluted with an equal volume of water and introduced onto an Amberlite XAD column which was eluted with methanol, and the eluate was derivatized to TMS ethers.

The analytical conditions for GC-ECD were: Hitachi 263-30 gas chromatograph; 3mm i.d.×1m glass column packed with 2% OV-17 on 80 to 100 mesh Gas Chrom Q; column temperature, 200°C; nitrogen flow rate, 55ml/min; and detector and injection temperatures, 290°C. The conditions for GC-MS (MF) were: JEOL QH-100 mass spectrometer, 2mm i.d.×1m glass column packed with 1% OV-17 on 80 to 100 mesh Gas Chrom Q, column temperature programed from 183 to 270°C at 8°C/min; helium flow rate, 40ml/min; ion source temperature, 300°C; and ionizing voltages, 20 and 70eV. The fragment ions monitored by GC-MS (MF) were m/z 512 (M+), 497, 422 and 407 for DON-TMS, and m/z 496 (M+), 481, 406 and 391 for DOM-1-TMS. The percentage recoveries of DON (tR, 5.2min) and nivalenol from cows milk were 67.2% and 58.1%, respectively, at a level of 10ng/ml, as determined by GC-ECD.

Although a trace amount of DON was detected in only a few urine and feces samples by GC-ECD and by GC-MS (MF), an unknown peak (tR, 3.2min) not found for control samples appeared on GC-ECD. On GC-MS (MF), the new peak gave fragment ions at m/z 496, 481, 407 and 406 and 391, each of which was shifted by 16 mass units (one oxygen atom) compared with in the case of DON-TMS. Moreover, the relative abundances of these ions were approximately constant in individual samples, suggesting that a common metabolite, i.e., DOM-1 (de-epoxidized DON), could be present in these samples.

In order to confirm this, extracts of cow excreta, plasma and milk were further cleaned up on silica gel thin-layer plates with a solvent system of chloroform–acetone (3:2,
and a band corresponding to DOM-1 was scraped off and eluted with methanol. As shown in Fig. 1, DOM-1 was clearly detected in cow urine, plasma and milk. Finally, the metabolite was identified as 3α,7α,15-trihydroxytrichothec-9,12-dien-8-one (i.e., DOM-1) by GC-MS (Fig. 2). The concentration of DOM-1, tentatively estimated by an external standard method, ranged from 150 to 4000 ng/ml in urine and from 6 to 20 ng/ml in milk, and was around 10 ng/ml in selected plasma samples. Quantitative analyses of DON and DOM-1, and the time course of elimination in the cow samples will be reported elsewhere (L. M. Cote, A. M. Dahlem, T. Yoshizawa, S. P. Swanson and W. B. Buck, submitted for publication).

The data presented here indicate that DON orally administered was de-epoxidized, and DOM-1, as a free metabolite, was transmitted into the cows milk through the blood circulation. It has already been reported that DON was de-epoxidized in vitro in rats and mice to afford DOM-1, which was found in the plasma and liver as well as the excreta.7) T-2 metabolites were also de-epoxidized in vivo in rodents.9) Recently, Yoshizawa et al.10) reported the in vitro de-epoxidation of DON and several other 12,13-epoxytrichothecenes by the intestinal microflora of mice. The metabolite, DOM-1, was also formed in vitro in rumen fluids of a lactating cow.11) This evidence suggests

![Fig. 1. Gas Chromatograms of Trimethylsilylated Extracts of (A) Urine (Day 6), (B) Plasma (Day 5) and (C) Milk (Day 5) of Lactating Cows Fed with a Ration Spiked with Deoxynivalenol.](image)

Each extract was cleaned up on a thin-layer plate and derivatized to trimethylsilyl ethers.

![Fig. 2. Mass Spectra of the Trimethylsilyl Ethers of DOM-1, a Deepoxidized Deoxynivalenol, Found in Biological Samples from Lactating Cows.](image)

A, DOM-1 (standard); B, feces; C, urine; D, milk.

* Base peak.
that DON orally administered is de-epoxidized into DOM-1 in the intestinal and/or rumen tracts, absorbed or reabsorbed, and then distributed into the blood, tissues and milk. Therefore, DOM-1 appears to be an appropriate diagnostic index for estimating the intake of DON in some animals. From the viewpoint of potential human exposure to the residual DOM-1 in dairy products, further extensive studies are required to clarify the metabolite contamination level in milk and also toxicological evaluation of the metabolite.

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