Fusariotoxicosis from Barley in British Columbia II. Analysis and Toxicity of Suspected Barley

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ABSTRACT

Fusariotoxin T-2, a trichothecene, was tentatively identified in barley samples which caused field outbreaks of mycotoxicosis in British Columbia. Geese died when fed the contaminated barley experimentally but mice were little affected after long term feeding. The methods used in the laboratory for trichothecene extraction and identification of T-2 toxin are described.

RÉSUMÉ

Les auteurs ont procédé à une identification présomptive de la fusariotoxine T-2, une trichotécine, à partir d'échantillons de l'orge responsable des cas de mycotoxicose survenus en Colombie britannique. L'alimentation expérimentale d'oies avec de cette orge entraîna leur mort; des souris ne manifestèrent toutefois que peu de changement, en dépit d'une alimentation expérimentale prolongée. Les auteurs décrivent aussi les méthodes de laboratoire qu'ils utilisèrent pour extraire la trichotécine et identifier la toxine T-2.

INTRODUCTION

Fusariotoxin T-2 is a 12, 13-epoxy-trichothecene mycotoxin produced in large quantities, often at low temperatures by certain *Fusarium* spp (7).

Outbreaks of suspected T-2 toxicosis occurred in British Columbia in October 1973 which involved domestic ducks, geese, horses and swine (5). The cause was attributed to barley known to have overwintered in the field in the Peace River District of northern Alberta/British Columbia.

This paper describes the methods of isolation and tentative identification of T-2 toxin in barley samples and the effects of feeding this barley to geese and mice.

MATERIALS AND METHODS

Isolation of Trichothecenes

Extraction — The suspect barley, a nontoxic barley sample and a nontoxic barley sample to which authentic T-2 toxin had been added, were extracted by the following combinations of procedures described by Hsu *et al* (6).

100 g of finely ground barley was shaken for one hour with 500 ml of ethyl acetate. The solvent was removed by filtration and retained. The extraction was repeated twice with further 500 ml portions of ethyl acetate. The extracts were combined and evaporated to an oily residue using a rotary evaporator.

The oily residue was dissolved in 100 ml 80% methanol and the lipids separated by extracting twice with 75 ml portions of hexane. The methanol fraction was diluted with 60 ml water to give a 50% methanol mixture. The pH was adjusted to 9.0 with saturated sodium carbonate solution and extracted twice with 100 ml portions of chloroform/ethyl acetate (50%, V/V). The combined extracts were evaporated in a

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rotary evaporator to give a light oily residue.

The residue was redissolved in a minimum volume of toluene/ethyl acetate (3:1, V/V) and further purified by passing through 15 g silica gel (0.05-0.20 mm) in a 20 x 400 mm column. The T-2 toxin was eluted with 250 ml toluene/ethyl acetate (3:1, V/V). The eluate was evaporated to a small volume using a rotary evaporator, transferred to a small vial and evaporated to dryness with a current of warm air. The residue was redissolved in 0.5 ml ethyl acetate by agitation.

Characterization — The three extracts were examined by thin layer chromatography (T.L.C.).

Known quantities of the sample extracts (1.0 to 10.0 μl), authentic T-2 toxin controls (2.5 to 10.0 μl of a solution containing 100 ppm T-2) and sample extracts with added authentic T-2 toxin (5.0 μl of 100 ppm T-2) were spotted on 0.25 mm thick, 20 x 20 cm silica gel G T.L.C. plates. The chromatograms were developed in ethyl acetate/toluene (3:1, V/V) as described by Burmeister (1). When the solvent had reached a line 15 cm from the spotting line the plates were removed from the developing tank and air dried.

The spots were visualized by long wave ultraviolet light after spraying the plates with 30% sulphuric acid in ethanol and charring at 120°C for 15 to 20 minutes (4). The T.L.C. was repeated using five different solvent systems (8).

Dermal tests—The extracts and controls were further examined using a skin test for trichothecenes: 2.0 μl of each sample were spotted on the shaved back of a white guinea pig, as described by Wei *et al* (10) for improved sensitivity to T-2 toxin.

Experimental Studies

Goose feeding experiment—Four young, mature geese were housed in pairs. Test group A (birds #2 and 3) were given free access to the suspect barley which they refused to eat and they were force fed. Goose #2 received a single dose of three ounces and goose #3 received a single dose of four ounces.

The control geese, group B, were fed a commercial feed *ad libitum*. Control goose #1 accidently escaped into test pen A, where it remained for several hours before being returned to pen B. Goose #4 remained as a control until the other geese had died. It was later used in an extended feeding trial using a lower dose and force-fed barley as follows: one ounce once daily for four days, no dose for two days, one ounce daily for three days.

Mouse feeding experiment — Three groups of three white mice were used. Group 1 acted as a control and was fed commercial feed, while groups 2 and 3 were fed the suspect barley *ad libitum*. Group 2 received whole grain and group 3 received the same grain which was ground to prevent them from selecting only good kernels. The test was continued for 77 days, after which time all the mice were sacrificed.

Tissues from all the geese and mice were examined bacteriologically and histologically, using routine methods.

RESULTS

Toxicological Findings

Extraction and characterization — The extracts from the suspect barley and barley sample with the added T-2 toxin on T.L.C. plates showed spots at identical relative flow (Rf) positions to the authentic T-2 toxin but showed no other aberrations from the extracts of the nontoxic barley. The nontoxic barley sample showed no spot at the T-2 toxin position. Quantitatively, when compared with the authentic T-2 toxin standards, the barley sample with added T-2 toxin indicated a recovery rate of 50%. The level of assumed T-2 toxin in the suspect barley when corrected for percent recovery was approximately 25 ppm. The detection limit of the method was approximately 2.5 ppm.

Dermal tests — The guinea pig used for the skin test was examined 24 hours after application of the samples. At this time the controls had produced reddish wheals of varying sizes, those produced by the higher levels of toxin being larger and having a whitish centre. The negative control and nontoxic barley extract produced no reaction. The suspect barley extract had produced a large reddish wheal with a whitish centre, comparable in size to the

highest control. This biological test also indicated a T-2 toxin equivalent of 25 ppm.

Clinical Observations

Goose #1 developed head tremors and died 24 hours after it had escaped into the test pen A.

Geese #2 and #3 developed tremors of the head and legs and both died within 19 hours of being force fed.

Goose #4 showed no clinical signs while being used as a control but during the subsequent extended feeding trial the following signs were observed: *third day* — excess water was consumed, droppings were greenish in colour, temperature 106.8°F, *sixth day* — feathers appeared ruffled, *seventh day* — slightly listless, *eighth day* — inactive, unsteady on legs, *ninth day* — slightly listless, not moving around freely, *tenth day* — reluctant to move, followed by death.

The mice fed the suspect barley showed a deterioration of the hair coat, whereas the control mice appeared normal.

Gross Pathological Findings

Goose #1 had a watery fluid in the lower esophagus. There was a severe necrosis of the proventriculus and gizzard as well as an intense enteritis.

In geese #2 and #3 the gizzards were filled with barley. The lining of the gizzards had a wet, shredded appearance, while the proventriculus appeared normal.

In goose #4 the entire digestive tract from the epiglottis to the gizzard contained barley. The esophageal wall was greatly distended as was the proventriculus. Necrosis of the mucous membrane was severe and began very high in the esophagus and continued into the gizzard. There was a blackened spot in both lungs of about one half inch in diameter.

No gross findings were observed in the mice.

Histopathological Findings

Goose #1 revealed the following abnormalities:

Liver — many areas of focal necrosis and cellular invasion.

Kidney — blood vessels injected.

Duodenum — extremely heavy inflammatory cell invasion of the lamina propria, degeneration and blood vessel congestion of the villar tips.

Gizzard — a sloughing of the keratinized surface epithelium had occurred and focal and diffuse inflammatory cell invasion was seen throughout the laminal propria.

Geese #2 and #3 both showed severe blood vessel congestion in the liver and kidney and there was a necrosis of the mucous membranes of the gizzards. The proventriculus and esophagus appeared normal.

Goose #4: histopathology of the blackened areas of the lungs revealed heavy mycellial growth, the kidney showed some blood vessel congestion. The esophagus, proventriculus and gizzard all exhibited severe necrosis of the mucous membranes and cellular invasion of the submucous areas.

The control mice (Group 1) showed no pathological changes. In the mice fed suspect barley (Groups 2 and 3) only the liver showed pathological changes, which included periacinar necrosis, nuclear and hepatocytic degeneration, fatty degeneration and an increase in extramedullary hemopoesis.

Bacteriological Findings

Goose #1. Gut content yielded a heavy growth of hemolytic and nonhemolytic *E. coli.*

Geese #2 and #3. Heavy growths of nonhemolytic *E. coli* were isolated from the lungs and spleens.

Goose #4. Light growths of nonhemolytic *E. coli* were cultured from the liver and kidney.

DISCUSSION

An outbreak of fusariotoxicosis is described in a companion paper (5). The absence from the spoiled barley of aflatoxins, citrinin, ochratoxins and zearalenone was confirmed by chemical analysis (5).

Although this laboratory did not have appropriate standards available for comparison, it is very probable that all known trichothecenes would have been extracted by the procedure described.

This assumption is substantiated by the finding that no toxins, other than the assumed T-2 trichothecene, showed on the T.L.C. plates at defined Rf values (8, 9). Nevertheless, the tests conducted do not eliminate the possibility that other unknown mycotoxins were also present in the barley. Neither do they conclusively prove that the compound detected was in fact T-2 toxin.

However, the tests performed did reveal the presence of a highly necrotic agent of mycotic origin, similar if not identical to T-2 toxin.

Although three of the test geese refused to eat the barley, one goose did consume a lethal dose and this shows that a field outbreak of toxicosis could have been produced by this barley. The outstanding lesion common to each test goose and to those poisoned in the field (5) was the severe necrosis affecting the digestive tract. From the extended feeding trial on goose #4 it seems likely that paralysis of the digestive tract may have occurred because the barley had not passed through the digestive tract at a normal rate.

Neural disturbances caused by T-2 toxin have been reported in chickens (11) but references were not found which describe the observed head tremors and leg muscle tremors in geese. Other tremorgenic mycotoxins have been isolated and described (2, 3, 12).

The finding that no mice died nor showed significant pathologic - anatomic changes after long term feeding of contaminated barley may indicate that these animals are of little or no value in fusario toxin feed tests.

Since submitting this manuscript for publication we have received a report from another laboratory, working with the same barley, which has failed to confirm the presence of T-2 toxin. Some components, not regularly detected in grain have been found that may be trichothecene mycotoxins not as yet characterized. This would explain some of the findings described which do not appear to be typical of T-2 toxicosis, particularly the lack of hemorrhaging in any of the animals, the tremors in the geese and the failure of the mice to show acute toxicosis.

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