Subacute Toxicity of Dietary 3-Acetyldeoxynivalenol in Mice

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ABSTRACT

3-Acetyldeoxynivalenol was incorporated into a semisynthetic diet at levels of 2.5, 5, 10 or 20 ppm and fed to mice for up to 48 days. Body weights and feed consumption were determined, and blood samples for hematological evaluation were taken. Selected tissues were examined microscopically and the humoral immune response was assessed using the Jerne plaque assay. 3-Acetyldeoxynivalenol caused a dose-related depressed feed consumption within the first seven days and reduced body weight until day 14 when fed at levels up to 10 ppm. When fed at a level of 20 ppm, an initial depression in body weight gain and a general malaise were followed by a return to normal. At necropsy, no macroscopic or microscopic lesions could be found. The immune response was not significantly affected after seven or 14 days, but at 21 days, a dose-dependent enhanced response was observed.

The findings indicate that, after an initial period of reduced feed intake, animals are apparently able to overcome the toxic effects of 3-acetyldeoxynivalenol.

Key words: Trichothecenes, deoxynivalenol, pathology, immunotoxicology, mycotoxins, vomitoxin.

RÉSUMÉ

Cette expérience consistait à incorporer 2.5,5,10 ou 20 ppm de 3-acétyldésoxynivalénol à une diète semi-synthétique destinée à des souris et à leur en donner sur une période qui allait jusqu'à 48 jours. On détermina le poids corporel, ainsi que la quantité de nourriture consommée, et on préleva des échantillons de sang en vue d'un examen hématologique détaillé. On procéda à l'examen microscopique de certains tissus et on utilisa la méthode des plages de Jerne, pour vérifer la présence d'anticorps sériques. La toxine précitée causa une réduction de la consommation de nourriture, proportionnelle à sa concentration, durant les sept premiers jours de l'expérience; à la concentration de 10 ppm, elle provoqua une diminution du poids corporel, jusqu'au 14^{e} jour. La dose de 20 ppm entraîna une diminution initiale du gain de poids corporel et un malaise général transitoires. Ni la nécropsie ni l'histopathologie ne révélèrent de lésions. La réaction immunitaire ne subit pas d'influence significative, après sept ou 14 jours; elle se révéla toutefois proportionnelle à la dose de toxine, au bout de 21 jours.

Ces constatations indiquent qu'après une réduction initiale de leur consommation de nourriture, les souris semblent réussir à surmonter les effets toxiques du 3-acétyldésoxynivalénol.

Mots clés: trichothécènes, désoxynivalénol, pathologie, immunotoxicologie, mycotoxines, vomitoxine.

INTRODUCTION

Deoxynivalenol (3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) (DON or vomitoxin) is a normocyclic trichothecene which is approximately ten times less potent in its toxic properties compared to T-2 toxin (1). After having determined the pathology of acute toxicity of 3-acetyldeoxynivalenol (3-AcDON) (2), it appeared to be prudent to extend the investigation to a subacute dietary toxicity study and to compare the results with those obtained with similar studies using T-2 toxin (3, 4).

MATERIALS AND METHODS

MICE

For the 21-day feeding trial (Experiment 1), weanling male outbred albino mice [Crl:CD1 (ICR) BR] (Charles River Canada Ltd., Laprairie, Quebec) were housed four to five per cage in stainless steel, screen-bottomed cages. For the 48-day feeding trial (Experiment 2), weanling male outbred albino Swiss mice (Animal Resources Centre, University of Saskatchewan) were housed five per cage in stainless steel, screen-bottomed cages. The animal facility was maintained at 21°C with a 12 h/12 h diurnal/nocturnal light cycle. All treatments and procedures conformed with the guidelines of the Canadian Council on Animal Care.

TOXIN

3-Acetyldeoxynivalenol (m.p. 184-186° C) was isolated from liquid cultures of *Fusarium graminearum* in the Chemistry and Biology Research Institute, Agriculture Canada (5).

DIET

For experiment 1, a modification of the American Institute of Nutrition (AIN-76) semipurified diet for the rat or mouse was used (see Table I). Crystalline 3-AcDON dissolved in 95% ethyl alcohol (10 mg/mL) was mixed with 300 mL of tap water per kg of diet to provide levels of 2.5, 5 or 10 ppm on a dry weight basis. The volume of ethyl

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TABLE I. Semi-synthetic Mouse Diet

Composition	%
Casein[a]	18.0
DL-methionine[a]	0.3
Choline bitartrate[a]	0.2
Cornstarch[b]	25.0
Dextrose[b]	39.0
Cellulose[a]	5.0
Corn oil[b]	5.985
Ethoxyguin[c] (50%)	0.015
AIN vitamin mixture 76[d]	1.0
AIN mineral mixture 76[d]	3.5
Gelatin[a] (Type I from swine skin)	2.0

[a]Sigma Chemical Company, St. Louis, Missouri

[b]Canada Starch Company Incorporated, Montreal-Toronto-Vancouver

[c]Dawe's Laboratories of Canada Ltd., Calgary, Alberta

[d]Nutritional Biochemicals, Cleveland, Ohio

Vitamin mixture components (1 kg): thiamine HCl, 600 mg; riboflavin 600 mg; pyridoxine HCl, 700 mg; nicotinic acid, 3 mg; d-calcium pantothenate, 1.6 mg; folic acid, 200 mg; d-biotin, 2 mg; cyanocobalamin, 1 g; retinyl palmitate pre-mix, 800 mg; d1--tocopheryl acetate pre-mix, 20 g; cholecalciferol, 2.5 mg; menaquinone, 5 mg; sucrose, finely powdered, 972.9 g

Mineral mixture components (1 kg): calcium phosphate, dibasic ($CaHPO_4$), 500 g; sodium chloride (NaCl), 74 g; potassium citrate, monohydrate ($HOC(COOK)(CH_4COOK)_4.H_2O$), 220 g; potassium sulfate (K_2SO_4), 52 g; magnesium oxide (MgO), 24 g; manganous carbonate (43-48% Mn), 3.5 g; ferric citrate (16-17% Fe), 6 g; zinc carbonate (70% ZnO), 1.6 g; cupric carbonate (53-55% Cu), 0.3 g; potassium iodate (KIO_3), 0.01 g; sodium selenite ($Na_2SeO_3.5H_2O$), 0.01 g; chromium potassium sulfate ($CrK(SO_4)_2.12H_2O$), 0.55 g; sucrose, finely powdered, 118 g

alcohol (1 mg/kg diet) was equilibrated for all diets and an equal volume of ethyl alcohol alone was added to the control diet. The diets were thoroughly mixed using a commercial mixer (Hobart Manufacturing Co. Ltd., Don Mills, Ontario) and extruded into pellets approximately 3 cm long by 1 cm in diameter. The diet was then dryed in a convection oven at low temperature (35° C) for 72-96 hours and stored at 4° C. During an acclimatization period of one week, all animals received a natural ingredient diet. On experimental day 0, the respective treatment groups were given the appropriate semisynthetic diets. The mice used in experiment 2 were treated in like fashion but were fed the semipurified diet containing levels of 0, 2.5, 10 or 20 ppm 3-AcDON.

HEMATOLOGY

At various times during experiment 1 (see experimental design) blood was collected from the tail vein for the following determinations: red and total white cell counts, differential white cell counts, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and reticulocyte counts (Coulter-S, Coulter Electronics Inc., Hialeah, Florida).

IMMUNOLOGICAL STUDIES

In experiment 1, the humoral immune response was assessed using the Jerne plaque assay (6). Following 7, 14 or 21 days exposure to the toxin, the immune response against sheep red blood cells (SRBC) was determined. The SRBC were injected i.p. in a 10% suspension (0.25 mL/mouse) on days 2, 9 and 16 for the respective studies. Background plaque counts were obtained from mice injected with 0.25 mL of saline rather than SRBC. Following cervical dislocation, each mouse was washed with 95% ethanol. The spleen was removed using sterile technique and placed in about 1 mL of cold Hank's medium (Grand Island Biological Co., Grand Island, New York) — pH 7.2. The spleen cells were teased from the splenic connective tissue using sponge forceps. Cellular debris was removed and spleen cell suspensions containing 3 x 10^6 lymphocytes/mL were prepared. The cell viability was determined using the trypan blue exclusion technique (8).

The spleen cell suspensions from individual mice were evaluated for their plaque-producing capability. The components of a single assay included: 20 μL of SRBC (25% suspension in Hank's media containing 5% fetal calf serum), 20 μL of guinea pig complement, 60 μL of Hank's media containing 5% fetal calf serum (all reagents: Grand Island Biological Co., Grand Island, New York) and 100 μL of spleen cell suspension. The mixture was incubated at 37° C for one-half hour as a monolayer suspension to facilitate plaque production. Each plaque or area of SRBC-lysis was enumerated visually under fluorescent light against a dark background.

EXPERIMENTAL DESIGN

In the first experiment, 13 mice per treatment were fed diets containing one of 0, 2.5, 5 or 10 ppm (w/w) 3-AcDON for either 7, 14 or 21 days. Body weights were taken on experimental day 0 and weekly thereafter.

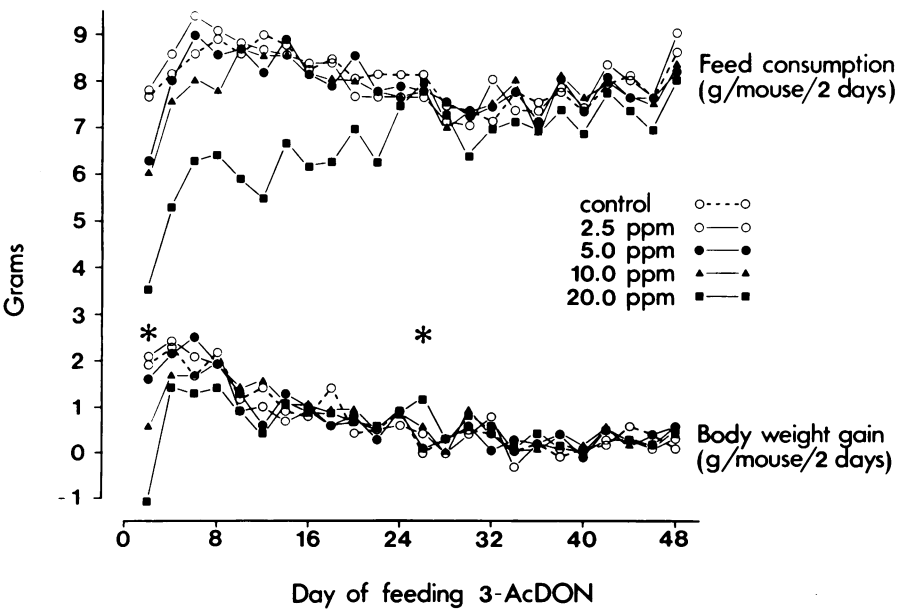

Fig. 1. Feed consumption and body weight gains, experiment 2. * indicates probability of no treatment differences ≤ 0.001.

TABLE IIa. Feed Consumption[a] (g/mouse/day), Experiment 1

Group	Week 1	Week 2	Week 3
Control	4.3[b] ± 0.1 (9)	3.9 ± 0.1 (6)	3.9 ± 0.2 (3)
2.5 ppm	4.0[c] ± 0.1 (9)	3.7 ± 0.1 (6)	3.5 ± 0.1 (3)
5 ppm	3.9[c] ± 0.1 (9)	3.7 ± 0.1 (6)	3.5 ± 0.1 (3)
10 ppm	3.4[d] ± 0.1 (9)	3.7 ± 0.2 (6)	3.6 ± 0.1 (3)
p[e] ⩽	0.00005	0.429	0.291

TABLE IIb. Body Weight[a] (g), Experiment 1

Group	Day 0	Day 7	Day 14	Day 21
Control	25.4 ± 0.3 (39)	28.6[b] ± 0.4 (39)	30.7[b] ± 0.5 (26)	32.0 ± 0.8 (13)
2.5 ppm	25.2 ± 0.3 (39)	27.8[b,c] ± 0.4 (39)	29.5[b,c] ± 0.5 (26)	31.9 ± 0.9 (13)
5 ppm	24.6 ± 0.4 (39)	27.6[b,c] ± 0.4 (39)	28.5[c] ± 0.5 (26)	32.2 ± 0.5 (13)
10 ppm	25.7 ± 0.3 (39)	26.6[c] ± 0.4 (39)	28.4[c] ± 0.6 (26)	31.9 ± 0.6 (13)
p ⩽	0.105	0.008	0.014	0.989

[a]Mean ± SE with number of observations in parentheses
[b,c,d]Means with different superscript letters are significantly different from one another ($p \leqslant 0.05$)
[e]Probability of no treatment differences

Feed consumption rates were determined daily by estimating the consumption per mouse based on four or five mice per cage per week. Blood samples were taken for hematological evaluation on days 7, 14 and 21 from five mice per group just prior to necropsy. These same animals were then killed by cervical dislocation and necropsies were performed. Selected tissues (thymus, spleen, stomach, duodenum) were collected, fixed in formalin, and routinely processed to obtain hematoxylin-eosin stained sections. The remaining eight mice in each group were used for the immunological function studies at each time (7, 14 and 21 days).

A second experiment was conducted using levels of 0, 2.5, 5, 10 or 20 ppm 3-AcDON, but ten animals each were fed the diets for 48 days. In this study, water and food consumption were determined every second day, and at necropsy only organ weights of spleen, thymus, liver and kidney were determined.

STATISTICAL EVALUATION

Body weight, feed consumption, hematological and plaque assay data were evaluated by means of ANOVA with treatment means being segregated using the Student-Newman-Keul's procedure with $p \leqslant 0.05$ (7).

TABLE III. Effect of Feeding 3-AcDON on Primary Immune Response[a] in Male CD-1 Mice[b] (Jerne Plaque Assay)

Concentration of Vomitoxin in Feed (ppm)	Days of Exposure	PFC/10^6 Spleen Cells[b]	Percentage of Control	p ⩽
0	7	451 ± 62[c] (6)	100	0.642[d]
2.5	7	492 ± 81 (7)	109	
5	7	425 ± 47 (7)	94	
10	7	385 ± 49 (7)	85	
0	14	381 ± 74 (7)	100	0.620
2.5	14	501 ± 74 (7)	131	
5	14	418 ± 49 (7)	110	
10	14	464 ± 71 (7)	122	
0	21	287[e] ± 32 (7)	100	0.026
2.5	21	361[e,f] ± 48 (6)	126	
5	21	425[e,f] ± 63 (7)	148	
10	21	498[f] ± 41 (7)	174	

[a]IgM or I9S antibody response against sheep red blood cells
[b]Plaque forming cells (antibody producing cells) per 10^6 spleen cells
[c]Mean ± SE with number of observations in parentheses
[d]Probability of no treatment differences among PFC/10^6 spleen cells
[e,f]Means with different superscript letters are significantly different from one another ($p \leqslant 0.05$)

Where appropriate, the resulting probability of the null hypothesis being true is quoted as a result of the ANOVA procedure, with $p \leqslant 0.05$ taken as a statistically significant difference. However, for the body weight gain data from experiment 2 (Fig. 1), since 24 contrasts were made over the feeding period, the value of p taken as an acceptable probability for a statistically significant difference was reduced to $p \leqslant 0.001$.

Since the data for feed and water consumption from experiment 2 were based on only two cages of animals per treatment, no statistical contrasts were applied.

RESULTS

In experiment 1, a dose-related reduction of feed consumption by day 7 was indicated, but not at days 14 or 21 (Table IIa). Body weights were depressed in the 10 ppm group at day 7, in the 5 and 10 ppm groups at day 14, but not at 21 days (Table IIb). All hematological aspects were found to be within the normal range, and neither postmortem nor microscopic examinations revealed any changes. The primary immune response against SRBC in mice exposed to 3-AcDON was not significantly affected after 7 or 14 days of exposure (Table III). The responses after 21 days (Table III), however, were enhanced in a dose-dependent fashion.

In the second experiment, all animals in the 0, 2.5, 5 and 10 ppm groups were clinically normal, whereas mice on the 20 ppm diet became dull and depressed-looking, with rough hair coats initially. Mice exposed to 20 ppm lost weight over the first two days of feeding (Fig. 1) and gained significantly less weight at this time than the other three groups, as did the 10 ppm group ($p \leqslant 0.00005$). A spurious result in weight gain occurred at day 26 with the 20 ppm-group having the greatest gain (Fig. 1) as compared to the other three groups, and the 10 ppm-group had a greater gain than the controls ($p \leqslant 0.00005$). Water consumption was consistently within normal limits among all groups and feed consumption is illustrated in Figure 1. All organ weights were within normal range, with the exception of the livers in the

TABLE IV. Effect of Feeding 3-AcDON on Organ Weights (g/100 g body weight[a], ± SE) of Male Swiss Mice, Experiment 2

Concentration of Vomitoxin in Feed (ppm)	Spleen	Thymus	Kidney	Liver
0	0.68 ± 0.06	0.16 ± 0.02	1.58 ± 0.04	5.53[b] ± 0.19
2.5	0.65 ± 0.06	0.15 ± 0.02	1.63 ± 0.03	5.66[b] ± 0.20
5	0.49 ± 0.02	0.16 ± 0.03	1.55 ± 0.06	5.77[b] ± 0.16
10	0.55 ± 0.03	0.17 ± 0.02	1.48 ± 0.05	5.94[b] ± 0.12
20	0.69 ± 0.10	0.14 ± 0.02	1.63 ± 0.05	7.08[c] ± 0.21
p[d] ≤	0.129	0.893	0.148	0.0000014

[a]n = Ten mice in each dose group
[b,c]Means with different superscript letters are significantly different from one another ($p \leq 0.05$)
[d]Probability of no treatment differences

20 ppm group which were significantly greater (Table IV).

DISCUSSION

Although 3-AcDON caused a dose-related depressed feed consumption for the first seven days when fed at levels up to 10 ppm 3-AcDON, no macroscopic or microscopic lesions could be detected in mice fed up to 10 ppm. Liver weights of mice fed 20 ppm 3-AcDON were significantly higher at day 48 which can be interpreted as an expression of enhanced hepatic activity and biotransformation of the toxin, similar to observations in mice fed T-2 toxin (4). None of the features characteristic of trichothecene toxicosis, such as lymphopenia, elevated granulocyte numbers, thymic and splenic atrophy, or gastrointestinal lesions, that were seen after feeding 10 or 20 ppm of T-2 toxin (3,4), could be observed.

The immune response, as measured by the Jerne plaque assay, was not significantly affected after 7 or 14 days, but at 21 days, a dose-dependent enhanced response was observed. Specific cell populations which might have been affected were not identified, although it does appear that a prolonged exposure, exceeding two weeks, is necessary to cause a significantly enhanced response. This suggests that the effects of 3-AcDON are not primarily due to direct cytotoxicity, but rather 3-AcDON may cause subtle alterations in cell subpopulations, possibly by impairing or stimulating their production. The biological significance of this enhanced response has yet to be determined.

The findings of our experiments indicate that levels of up to 10 ppm 3-AcDON in the diet cause minimal deleterious effects if one disregards the negative effect on body weight gain in the first seven days. The conclusion is that 3-AcDON is considerably less toxic than other trichothecenes, as judged by the variables studied in the animal system (mice) used.

In a recently published study of the health problems in swine associated with feeding DON-contaminated grain in the U.S. (9), the mean concentration of DON in feed samples was 3.14 ppm (range 0.1 to 41.6 ppm), and it was found that the most frequently observed clinical signs were reproductive problems. This would suggest that one would have to consider more subtle effects other than vomition and failure to gain weight when assessing the potential toxicity of vomitoxin.

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