Pathology of Acute 3-Acetyldeoxynivalenol Toxicity in Mice

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ABSTRACT

Mice were killed 2, 4, 6, 12, 24, 48 and 96 hours after intragastrical administration of 0, 5, 10, 20, or 40 mg/kg body weight of 3-acetyldeoxynivalenol. The animals became clinically ill after 12 hours and some animals in the highest dose group died. Histological examination of duodenal crypts, thymus and spleen revealed, in all dose groups, presence of the characteristic lesions that are known to be produced by trichothecenes, but the intensity of lesions in the 40 mg group corresponded to lesions known to be caused by 4 mg/kg of T-2 toxin.

A rabbit skin bioassay with 3-acetyldeoxynivalenol gave negative results on one occasion and a mild reaction to 100 to 500 μg/mL on another.

It is concluded that 3-acetyldeoxynivalenol is considerably less toxic than T-2 toxin, but causes acute effects in the dividing cells of the body in a manner characteristic of trichothecenes.

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

RÉSUMÉ

Cette expérience consistait à administrer du 3-acétyldésoxynivalénol, directement dans l'estomac de souris, à raison de 0,5,10,20 ou 40 mg/kg, et à les sacrifier ensuite au bout de deux, quatre, six, 12,24,48 et 96 heures. Elles commencèrent à manifester des signes cliniques, au bout de 12 heures, et certaines de celles qui avaient reçu la plus forte dose de toxine moururent. L'examen microscopique des cryptes duodénales, du thymus et de la rate des souris des divers groupes expérimentaux révéla la présence des lésions pathognomoniques d'une intoxication par les trichothécènes; la gravité de celles qu'on retrouva chez les souris qui avaient reçu la plus forte dose de toxine correspondait à celle des lésions qui résultent ordinairement de l'administration de 4 mg/kg de toxine T-2.

L'application de la toxine expérimentale sur la peau de lapins donna d'abord des résultats négatifs, mais elle provoqua une légère réaction, lors d'une autre tentative où on utilisait des solutions dont la concentration de la toxine variait de 100 à 500 μg/mL.

Les résultats de cette expérience permirent de conclure que le 3-acétyldésoxynivalénol est beaucoup moins toxique que la toxine T-2, mais qu'il cause des effets aigus, caractéristiques des trichothécènes, sur les cellules en division de l'organisme.

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

INTRODUCTION

Deoxynivalenol (3, 7, 15-trihydroxy-12, 13-epoxytrichothec-9-en-8-one, DON or vomitoxin),a normocyclic trichothecene (1), is reported to cause feed refusal and emesis when young livestock and poultry ingest contaminated grain (2). The resulting reduction in body weight gains (growth) is well documented for pigs (3,4). Pigs have been reported to refuse vomitoxin-contaminated corn containing 0.7 ppm (5) or 1 ppm (6). Poultry are apparently more tolerant (7).

The LD_{50} values, in mice, for DON and its precursor, 3-acetyldeoxynivalenol (3-AcDON) are given as 70.0 mg/kg and 49.0 mg/kg, respectively, after i.p. injection and 46.0 mg/kg and 34.0 mg/kg *per os*; these values have to be compared with 5.2 mg/kg for T-2 toxin, another normocyclic trichothecene (1). There are no reports describing the pathology of acute deoxynivalenol toxicity.

The objectives of this study were to determine the extent and intensity of injury in duodenal crypts, spleen and thymus, to characterize the temporal development of the lesions after intragastric administration of the toxin, and to compare the findings with those obtained from T-2 toxin studies (8), in order to determine the relative toxicity. The selection of the organs to be investigated was governed by the experiences gained with the T-2 toxin studies (8).

In addition, we attempted to define the cutaneous irritancy potential of 3-acetyldeoxynivalenol, again in relation to other trichothecenes (9).

MATERIALS AND METHODS

MICE

Weanling male outbred albino mice [Crl:CDl (ICR) BR] (Charles River Canada Ltd., Laprairie, Quebec) were housed four to 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.

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TOXIN

3-acetyldeoxynivalenol (3-AcDON; m.p. 184-186° C) was isolated from liquid cultures of *Fusarium graminearum* by the Chemistry and Biology Research Institute, Agriculture Canada (10). For the acute toxicity studies, the toxin was dissolved in propylene glycol (Fisher Scientific Ltd., Edmonton, Alberta) in appropriate concentrations to deliver 1 mL of vehicle per 100 g body weight.

DIET

The mice were supplied *ad libitum* with a pelleted natural-ingredient diet (Department of Animal Science, University of Saskatchewan, Saskatoon) during an acclimatization period of at least one week. Food was withdrawn 16 hours before treatment. For the two, four and six hour evaluations, food was not returned. For the 12, 24, 48 and 96 hour evaluations, food was provided six hours after treatment.

EXPERIMENTAL DESIGN

After an overnight fast, mice were weighed and given 3-AcDON in propylene glycol or propylene glycol alone (control) through stainless steel animal feeding tubes (Popper and Sons, Inc., New Hyde Park, New York) introduced directly into the stomach. Five mice were used for each dose (0, 5, 10, 20 and 40 mg/kg body weight) for the 2, 4, 6, 12, 24, 48 and 96 hour periods, for a total of 175 mice. The mice were then killed at the predesignated time by cervical dislocation and complete necropsies performed.

Selected tissues (duodenum and spleen from the animals killed at two, four and six hours, and stomach, duodenum, lower jejunum, spleen, thymus, pancreas, liver and heart from the 12 to 96 hour animals) were collected, fixed in formalin and processed routinely to obtain hematoxylin-eosin stained sections. The sections were evaluated subjectively using quantitative descriptions. For cortical thymus, central thymus, splenic lymph follicles and splenic red pulp, the following descriptors were used: 0 = no necrotic cells, 1 = few necrotic cells, 2 = some necrotic cells, 3 = numerous necrotic cells, 4 = almost all cells necrotic, 5 = complete necrosis. For the duodenum, the number of mitotic figures (0 = no mitoses, 1 = a few, 2 = some, 3 = many, 4 = large number, to 5 = massive number) and the number of necrotic cells in crypts (0 = none, to 5 = massive numbers, numerous) were estimated.

For the skin bioassay, concentrations of 100, 200, 300, 400 and 500 μg/mL were applied to the skin of rabbits in 2 x 1 quantities, and the results were read 24 hours later (for details of method, see Hayes and Schiefer, 9). This procedure was repeated once.

STATISTICAL EVALUATION

The raw quantitative descriptor scores were compared among treatment groups for each time period using the Kruskal-Wallis test adjusted for tied ranks (11). Where the probability of the resulting Chi-square statistic was $\leqslant 0.05$, the multiple range procedure described by Daniel (12) was used to differentiate mean ranks, with $\alpha = 0.15$ (unlike values of α customarily encountered in parametric multiple comparison procedures, e.g. 0.01, 0.05).

The results of these analyses are reported in Tables I, II and III; however, as a descriptive statistic for each treatment group, the median was used.

TABLE Ia. Median Descriptor Scores of Duodenal Mitoses

Dose of 3-AcDON (mg/kg body weight)	Time to Sacrifice (hours)						
	2	4	6	12	24	48	96
0	5^{a}	5^{a}	5^{a}	5^{a}	5^{ab}	5	5^{a}
5	0^{b}	4^{ab}	5^{a}	4^{ab}	5^{b}	5	5^{a}
10	0^{b}	0^{b}	1^{ab}	5^{a}	5^{b}	5	5^{a}
20	0^{b}	0^{b}	0^{b}	2^{bc}	5^{ab}	5	5^{a}
40	0^{b}	0^{b}	0^{b}	0^{c}	2^{a}	5	3^{b}
$p^{d} \leqslant$	0.0005	0.0005	0.0005	0.0005	0.003	0.642	0.0005

[abc]Medians with different superscript letters are significantly different from one another ($p \leqslant 0.15$) within a column

[d]Probability of no treatment differences

TABLE Ib. Median Descriptor Scores of Necrotic Duodenal Cells

Dose of 3-AcDON (mg/kg body weight)	Time to Sacrifice (hours)						
	2	4	6	12	24	48	96
0	0^{a}	1^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
5	1^{ab}	1^{ab}	0^{ab}	1^{ab}	1^{a}	1^{ab}	0^{a}
10	1^{ab}	2^{b}	3^{bc}	1^{abc}	1^{a}	1^{ab}	1^{ab}
20	2^{b}	4^{b}	4^{bc}	4^{bc}	2^{ab}	2^{b}	1^{ab}
40	2^{b}	5^{b}	5^{c}	5^{c}	4^{b}	2^{b}	3^{b}
$p^{d} \leqslant$	0.010	0.014	0.001	0.001	0.002	0.023	0.017

[abc]Medians with different superscript letters are significantly different from one another ($p \leqslant 0.15$) within a column

[d]Probability of no treatment differences

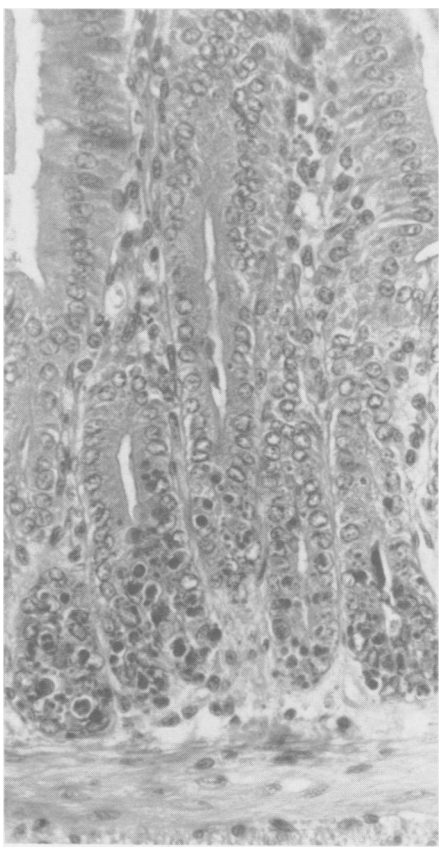

Fig. 1a. Duodenum, six hours after administration of 40 mg/kg of 3-AcDON. Numerous pyknoses in crypt cells are present. H & E. X250.

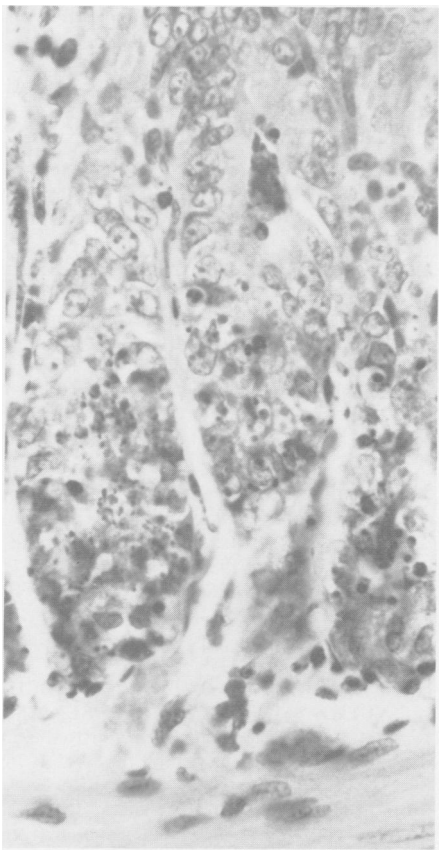

Fig. 1b. Duodenum, 12 hours after administration of 20 mg/kg of 3-AcDON. Karyorrhexis and accumulation of debris in crypts is very noticeable. H & E. X400.

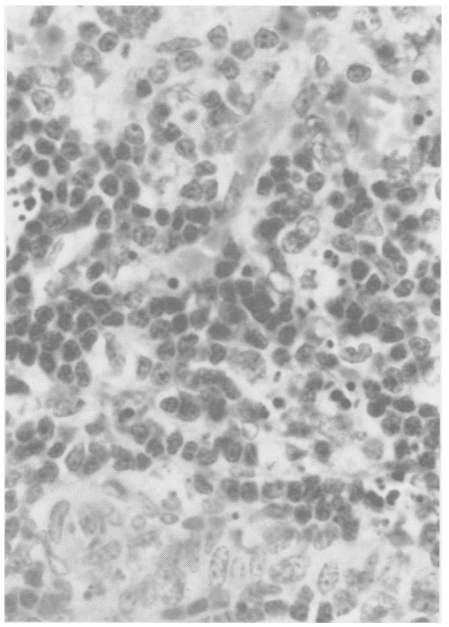

Fig. 2. Spleen, 12 hours after administration of 20 mg/kg of 3-AcDON. Follicle artery is in left lower corner; there is loss of cells of the follicle, and karyorrhexis can be seen. H & E. X400.

TABLE IIa. Median Descriptor Scores of Necrotic Cells in Splenic Lymph Follicles

Dose of 3-AcDON (mg/kg body weight)	Time to Sacrifice (hours)						
	2	4	6	12	24	48	96
0	0^{a}	0^{a}	0^{a}	0^{ab}	0^{ab}	1^{ab}	0^{a}
5	0^{a}	0^{a}	0^{a}	1^{ab}	0^{a}	0^{ab}	0^{a}
10	0^{a}	0^{a}	0^{a}	1^{ab}	0^{a}	-[c]	0^{a}
20	1^{ab}	0^{ab}	0^{ab}	2^{b}	0^{ab}	0^{a}	0^{a}
40	4^{b}	4^{b}	3^{b}	2^{b}	2^{b}	2^{b}	3^{b}
$p^{d} \leqslant$	0.002	0.001	0.002	0.0005	0.009	0.028	0.001

[ab]Medians with different superscript letters are significantly different from one another ($p \leqslant 0.15$) within a column

[c]Insufficient data (number of animals) for this concentration of 3-AcDon for this time period

[d]Probability of no treatment differences

TABLE IIb. Median Descriptor Scores of Necrotic Cells in Splenic Red Pulp

Dose of 3-AcDON (mg/kg body weight)	Time to Sacrifice (hours)						
	2	4	6	12	24	48	96
0	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	1^{ab}
5	1^{ab}	2^{ab}	2^{b}	1^{ab}	0^{a}	1^{ab}	0^{a}
10	1^{b}	1^{ab}	1^{ab}	2^{bc}	0^{a}	-[d]	0^{a}
20	2^{b}	2^{b}	1^{ab}	3^{bc}	1^{ab}	1^{ab}	0^{a}
40	2.5^{b}	5^{b}	3^{b}	4^{c}	3^{b}	1^{b}	2^{b}
$p^{e} \leqslant$	0.002	0.012	0.001	0.001	0.027	0.039	0.009

[abc]Medians with different superscript letters are significantly different from one another ($p \leqslant 0.15$) within a column

[d]Insufficient data (number of animals) for this concentration of 3-AcDon for this time period

[e]Probability of no treatment differences

This may be the cause of some confusion, where the results of a test applied to mean ranks attribute different qualities to medians of equal value. However, it is felt that mean ranks are potentially more confusing in describing the raw scores.

RESULTS

The animals remained clinically normal for up to 12 hours after application. After this time, they started to huddle in a corner of the cage and were reluctant to move about, particularly in the 20 and 40 mg groups. One animal of the 40 mg group died at 24 hours and another at 48 hours, and by 96 hours, all animals in the 40 mg group were dead.

The extent and intensity of duodenal crypt injury is illustrated in Tables Ia and Ib. By two hours, mitotic activity (Table Ia) was significantly reduced in all groups compared to controls; mitotic figures thereafter could be seen returning in increasing numbers dependent on dose of toxin given. Necrotic cells (Table Ib) appeared by two hours in increased numbers in the 20 and 40 mg groups compared to controls; by four hours, increased numbers were seen in the 10, 20 and 40 mg groups; by 96 hours, only the 40 mg group was elevated. Many pyknotic cells were evident by 12 hours (Fig. 1a), and they often formed into plugs of necrotic cells in the cryptal lumina (Fig. 1b). Multifocal necrosis, characterized by nuclear pyknosis and karyorrhexis (Fig. 2) occurred in the spleens by four hours and persisted up to 96 hours. The splenic lymph follicle response showed no significant effects over time, but there was a sustained increase in the 40 mg group, except for 24 and 48 hours (Table IIa). In the red pulp, scores peaked at 12 hours (Table IIb) in the 10, 20 and 40 mg groups. Similar lesions were seen in the lymphoid cells of the Peyer's patches of the intestine. The thymus was also affected (Fig. 3) and for the cortical thymus (Table IIIa) the main effects were an increase of scores in the 20 and 40 mg groups, compared to controls at 12 hours, and a sustained increase in the 40 mg group at 96 hours. In the medullary part of the thymus (Table IIIb), the scores were increased in all doses at 12 hours, and remained high in the 40 mg group up to 96 hours.

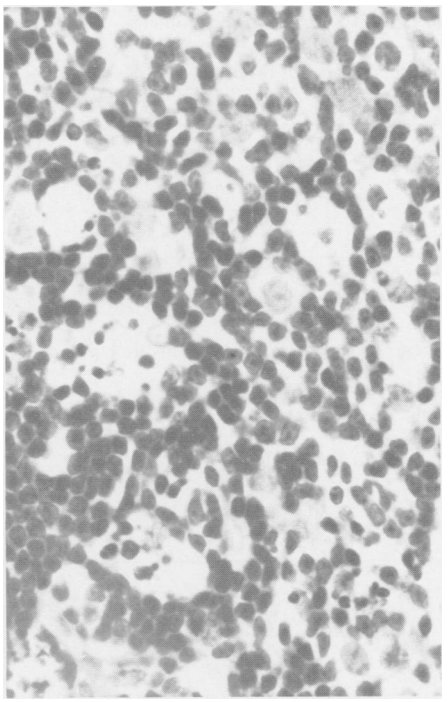

Fig. 3. Cortical thymus, 24 hours after application of 20 mg/kg of 3-AcDON. Note scalloping and presence of necrotic cells and cellular debris. H & E. X400.

No lesions were found in stomach, pancreas, liver or heart.

The skin bioassay results in the first trial were all negative, i.e. 3-AcDON did not produce any reactions. In the second trial 100 to 400 μg/mL of 3-AcDON gave a mild reaction, equal to 2.5 to 5.0 μg/mL of T-2 toxin (applied as standard); the 500 μg/mL 3-AcDON application gave a reaction comparable to 10 μg/mL of T-2 toxin.

DISCUSSION

Judging from the skin bioassay, 3-AcDON was considerably less irritant to skin than T-2 toxin, by a factor of 50 to 100 fold. Intragastrical application of 3-AcDON caused lesions which were similar to those induced by T-2 toxin (8), but in general it can be said that the intensity of the lesion in the 40 mg/kg 3-AcDON group was comparable to lesions caused by 4 mg/kg of T-2 toxin (8). Nevertheless, it should be kept in mind that 3-AcDON affected the general health status of the animals, and a dose of 40 mg/kg BW produced death by 24 hours, with all animals dying by 96 hours.

It can be concluded that 3-AcDON, while considerably less toxic than T-2 toxin, affects the dividing cells of the body in a manner characteristic for trichothecenes. Considering the fact that 3-AcDON is more toxic than DON (1), concerns with respect to dietary intake of vomitoxin appear to be less justified. Before making premature judgments on the basis of the acute toxicity of 3-AcDON, however, one should take into consideration the findings of a 21 day or longer feeding study, described in another paper (13). The fact that the rodent does not respond with vomition when vomitoxin is included in the diet has to be considered as well. The process of vomition and feed refusal observed in many other species will reduce even further the amount of toxin which reaches internal organs.

TABLE IIIa. Median Descriptor Scores of Necrotic Cells in Cortical Thymus

Dose of 3-AcDON (mg/kg body weight)	Time to Sacrifice (hours) 12	24	48	96
0	1^{a}	1^{a}	1^{a}	1^{a}
5	2^{ab}	1^{a}	1^{a}	0^{a}
10	3^{ab}	1^{a}	1^{a}	0^{a}
20	3^{b}	3^{ab}	1^{a}	1^{ab}
40	4^{b}	5^{b}	5^{b}	5^{b}
$p^{c} \leqslant$	0.003	0.003	0.025	0.027

[ab]Medians with different superscript letters are significantly different from one another ($p \leqslant 0.15$) within a column

[c]Probability of no treatment differences

TABLE IIIb. Median Descriptor Scores Necrotic Cells in Central Thymus

Dose of 3-AcDON (mg/kg body weight)	Time to Sacrifice (hours) 12	24	48	9c
0	0^{a}	0^{a}	0^{a}	0^{a}
5	2^{b}	1^{ab}	0^{a}	0^{a}
10	2^{b}	0^{a}	1^{ab}	0^{a}
20	2^{b}	1^{b}	1^{ab}	0^{a}
40	2^{b}	3.5^{b}	4^{b}	5^{b}
$p^{c} \leqslant$	0.031	0.024	0.003	0.036

[ab]Medians with different superscript letters are significantly different from one another ($p \leqslant 0.15$) within a column

[c]Probability of no treatment differences

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