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Developmental toxicity of fumonisin in Syrian hamsters

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Abstract. The effects of fumonisin on development of Syrian hamster fetuses were studied using fumonisin B_1 and B_2 extracted from *Fusarium moniliforme* corn-culture and purified fumonisin B_1. A significant increase in litters with fetal deaths occurred with the high doses of purified (18 mg FB_1/kg) and culture-extracted (18 mg FB_1 plus 4.5 mg FB_2) fumonisin. It is concluded that prenatal exposure to fumonisin on days 8 and 9 of gestation is detrimental to fetal hamster survivability but does not induce clinical maternal intoxication at these doses. Equivalent doses of fumonisin B_1, whether from culture-extract or pure solution produced similar results.

Key words: Developmental toxicity, Fetal toxicity, Fumonisin, *Fusarium moniliforme*, Hamster

Introduction

Fumonisin B_1 (FB_1) has been recognized as the causative agent of a variety of animal diseases including leukoencephalomalacia in horses [1, 2], hepatopathy and hepatocarcinoma in laboratory rats [3] and pulmonary edema in swine (PPE) [4]. Also, chronic exposure to dietary fumonisins induced nodular hyperplasia in the liver [5] and right ventricular enlargement secondary to medial hypertrophy of pulmonary arterioles in pigs [6]. It was suggested that fumonisin-contaminated corn contributed to abortions in pregnant sows [4]. However, abortions occured in sows recovering from PPE, and fetal losses may have been secondary to hypoxia resulting from maternal respiratory distress.

Recently, purified FB_1 and *Fusarium proliferatum* culture extract have been shown to be embryotoxic when inoculated into incubating eggs [7]. A previous study by the authors found that fetal deaths increased in hamsters when an aqueous extract containing known concentrations of fumonisin B_1 and B_2 was administered during the period of organogenesis [8]. The preparation used was not purified. Therefore, fetal anomalies could not be attributed to FB_1 directly. Also, a longer duration of treatment (3 to 5 days) presumably contributed to the number of fetal deaths. The present study compared the developmental effects of short-term administration of purified FB_1 or culture-extracted FB_1 and FB_2 solutions.

Materials and methods

Fumonisin. Fusarium moniliforme M-1325 (Pennsylvania State University Fusarium culture collection) used in these experiments has been shown to produce 4–6 g FB_1 and 1.2–1.9 g FB_2 per kg corn culture material. Preparation of the culture material has been described [9]. Ground culture material was extracted with water 4:1 and filtered under vacuum to provide an aqueous solution of fumonisin for oral administration.

Freeze-dried 98% pure fumonisin B_1 (Sigma Chemical, St. Louis, MO) was dissolved in distilled water to produce an aqueous solution of similar concentration to the culture extract. Both solutions were analyzed for FB_1 and FB_2 by HPLC [10]. Fumonisin B_2 was 25% (±4%) of the concentration of FB_1 in the culture material extract. Additional analysis of the corn-culture material failed to detect aflatoxin, T-2 toxin, ochratoxin A, zearalenone, vomitoxin, citrinin, sterigmatocystin, fusarin C and moniliformin (courtesy of G. Bennett, USDA-ARS, NCAUR, Peoria, IL).

Animals and experimental design. One hundred fifteen, date-mated, female, Syrian hamsters (Sasco, Omaha, NE) were used. The hamsters were cared for according the protocol approved by the University of Missouri Animal Care and Use Committee.[1] Hamsters

[1] Guide for the Care and Use of Laboratory Animals, NIH publication No. 86-23, 1985.

were housed individually under a 12 hour light-dark regimen and given feed and water ad libitum. The diet consisted of a commercial, pelleted laboratory animal feed (Ralston Purina, St. Louis, MO). Hamsters were received on the seventh day of gestation as determined by the presence of spermatozoa in vaginal swabs one day (day 1) after exposure to males. Females were randomly assigned to experimental groups.

Animals assigned to treatment groups 6EX, 12EX and 18EX were given 6.0, 12.0 and 18.0 mg FB_1 in culture extract/kg/d by gavage, respectively. Treatment groups 12P and 18P received 12.0 and 18.0 mg purified FB_1 in water/kg/d, respectively. Control group (C) females received volumes of water equivalent to those of aqueous fumonisin given to 18EX and 18P females. All treatment doses were administered on days 8 and 9 of gestation. The concentration of FB_1 in both aqueous solutions was approximately 1300 ppm. The concentration of FB_2 in culture extract was about 325 ppm.

Data collection. Females were weighed on days 8, 12 and 15 of gestation, and euthanized by CO_2 inhalation on day 15. Fifty-one females were pregnant. Gravid uterine weights were recorded and the number of implantations, normal fetuses, dead and resorbing fetuses and/or resorption sites were noted. Adjusted net gain for pregnant females was calculated by subtracting the weight of the gravid uterus from weight gained during the study. This minimized the variability due to fetal numbers and weights. Average fetal weight was calculated from the total weight of live, term fetuses per litter. Tissue samples of liver, kidney, uterus and placenta were obtained from each dam for histologic evaluation. These samples were fixed in formalin, embedded in paraffin, sectioned, mounted and stained with H&E. Serum was collected from 47 pregnant females for determination of aspartate aminotransferase (AST) and total bilirubin concentrations.

Fetal evaluations were performed by the procedures described in an earlier study [8]. Fetuses were examined for developmental maturity and for structural normalcy [11]. Crown-rump lengths were recorded and internal examinations were performed at a magnification of 10× under a dissecting microscope (Bausch and Lomb, Tampa, FL). Abnormal findings were recorded by litter.

Following the procedure described by Manson & Kang [12], 25% of fetuses per litter were eviscerated, cleared with 1–2% KOH and stained with alizarin red. After processing, fetuses were stored in glycerin and evaluated for skeletal integrity and calcification.

Statistics. Data associated with individual females or their litters were analyzed using various statistical methods. Effect of fumonisin on net maternal weight gain, average fetal weight, number of implantations, and AST and total bilirubin concentrations were evaluated by analysis of variance. Fetal crown-rump lengths were evaluated using a nested analysis of variance. Total fetal deaths (resorptions plus dead term fetuses) per litter were analyzed by Kruskal-Wallis nonparametric ranking [12]. Fisher's Exact test was used to analyze the incidence of litters containing one or more malformed fetuses. Pregnant females were classified by dose and mean responses were examined using a Least Significant Difference Rule with Type I error rate = 0.05. All computations were done using SAS Software provided by the University of Missouri-Columbia.

Results

Maternal toxicity. Oral administration of purified (P) fumonisin B_1 or culture extracted (EX) FB_1 and FB_2 at the doses reported here failed to induce clinical signs of maternal intoxication. Net maternal weight gain and serum AST concentrations for all treatment groups were not different from the control group values (Table 1). Total bilirubin level for group 12P was significantly below that for the control group, but similar to reported values for normal non-fasted female hamsters [13]. There were no dose-dependent differences noted for these variables. Histologic examination of formalin-fixed maternal liver, kidney and placenta revealed similar histologic changes as described previously [8].

Developmental toxicity. Effects of fumonisin on developing fetuses were expressed most often by fetal death and resorption. Dead fetuses were variably autolyzed and edematous at the time they were recovered from excised uteri. For purposes of comparison, total number of fetal losses per litter were calculated by adding the number of resorption sites and dead fetuses. The frequency of litters with fetal losses increased as the dose of fumonisin increased (Table 2). A significantly greater number of females in groups 18P (100%) and 18EX (75%) than in groups C (45%) and 12P (20%) had litters with one or more affected fetuses. Increased numbers of fetuses per litter were resorbed or dead for 18EX females, including 3 entire litters (Table 2).

Table 1. Parameters examined to assess maternal toxicity following fumonisin treatment[1]

Treatment group[2]	Weight gain	AST	Total bilirubin[3]
Control	29.09 ± 9.25 (11)	147.80 ± 192.81 (10)	0.54 ± 0.28[a] (10)
6EX	33.80 ± 12.26 (10)	150.44 ± 146.78 (9)	0.52 ± 0.32[a] (9)
12P	33.40 ± 8.08 (5)	59.80 ± 15.80 (5)	0.18 ± 0.04[b] (5)
12EX	20.29 ± 26.91 (7)	66.29 ± 33.93 (7)	0.47 ± 0.29[ab] (7)
18P	24.83 ± 7.31 (6)	94.40 ± 57.22 (5)	0.76 ± 0.13[a] (5)
18EX	21.00 ± 7.93 (12)	124.82 ± 71.52 (11)	0.68 ± 0.31[a] (11)

[1]Mean values ± SD (n); weight in grams, AST in international units per liter, bilirubin in milligrams per deciliter.

[2]mg fumonisin B_1/kg in aqueous extract (EX) or pure solution (P); extract also contains fumonisin B_2 at 25% FB_1 concentration.

[3] Values denoted by different letters are statistically different (< 0.05).

Table 2. Fetal expression of fumonisin exposure

Treatment Group[1]	Ave. rank for litter with one or more fetal deaths[2]	Frequency of litters with one or more fetal deaths	Frequency of dead fetuses
Control	19.82 ± 11.25[a]	5/11 (45%)	7/130 (5/4%)
6EX	28.15 ± 13.27[ab]	7/10 (70%)	16/125 (12.8%)
12P	13.80 ± 7.38[a]	1/5 (20%)	1/75 (1.3%)
12EX	22.21 ± 15.99[ab]	3/7 (43%)	16/96 (17.2%)
18P	33.33 ± 9.83[b]	6/6 (100%)	15/77 (19.5%)
18EX	33.50 ± 15.89[b]	9/12 (75%)	57/157 (36.3%)

[1]mg fumonisin B_1/kg in aqueous extract (EX) or pure solution (P); extract also contains fumonsin B_2 at 25% FB_1 concentration.

[2]Values denoted by different letters are statistically different ($p < 0.05$).

In addition to increasing numbers of fetal losses, an increase in the number of fetuses with external malformations was also identified (Table 3). This increase was not statistically significant, however, because malformed fetuses were grouped within a few litters. The most frequently identified abnormality was a hooked or curled tail (Fig. 1). One litter (14.3%) from group 12EX had 11 of 15 fetuses with curled tails. In group 18P, one of six litters (16.7%) contained 8 of 14 fetuses with hooked or curled tails. Eleven of the 14 exhibited ectrodactyly of the front and/or rear limbs and 2 had cleft palates. Group 18EX had 33.3% (4/12) litters with external malformations. Three litters contained 3 of 4, 5 of 8, and 12 of 15 term fetuses with hooked or curled tails. In two of these litters there were also 5 and 3 resorbed or dead fetuses. The fourth affected 18EX litter contained 3 of 4 term fetuses with ectrodactyly and cleft palates (Fig. 2) and 5 resorbed or dead fetuses. Internal organ and skeletal examinations did not reveal other developmental abnormalities.

Mean weights and crown-rump lengths for live term fetuses are listed in Table 4. Fetuses from 6EX females were heavier than fetuses from 12P, 18P and 18EX females, however, none were significantly different from control group fetuses. Live term fetuses from 18EX females had shorter crown-rump lengths than fetuses from 6EX, 12EX and C females. Fetuses from other treatment groups were not different from controls.

Discussion

Maternal intoxication was not induced at the doses of fumonisin used, as evidenced by measurement of net maternal weight gain, serum AST and total biliru-

Table 3. External malformations found in live term hamster fetuses on day 15 of gestation after prenatal exposure to fumonisin

Treatment group[1]	Frequency of fetuses with external malformations	Individual malformations found		
		Hooked or curled tail	Ectrodactyly	Cleft palate
Control	0/123	0	0	0
6EX	0/109	0	0	0
12P	0/74	0	0	0
12EX	11/77	11	0	0
18P	11/62	8	11	2
18EX	23/100	20	4	3

[1]mg fumonsin B_1/kg in aqueous extract (EX) or pure solution (P); extract also contains fumonisin B_2 at 25% FB_1 concentration.

Table 4. Mean fetal weights and crown/rump lengths for all treatment groups[1]

Treatment group[2]	Fetal weight[3]	Crown/rump length[3]
Control	1.84 ± 0.44^{ab} (11)	23.79 ± 2.58^{ab} (123)
6EX	2.22 ± 0.58^{a} (10)	25.26 ± 2.44^{a} (109)
12P	1.54 ± 0.11^{b} (3)	24.03 ± 3.30^{abc} (68)
12EX	1.84 ± 0.52^{ab} (5)	23.99 ± 2.64^{ab} (77)
18P	1.47 ± 0.36^{b} (6)	22.80 ± 2.38^{bc} (60)
18EX	1.47 ± 0.26^{b} (8)	21.68 ± 2.15^{c} (97)

[1]Mean values ±SD (n); weight in grams, length in millimeters.
[2]mg fumonisin B_1/kg in aqueous extract (EX) or pure solution (P); extract also contains fumonsin B_2 at 25% FB_1 concentration.
[3]Values within columns denoted by different letters are statistically different ($p < 0.05$).

bin levels. Fumonisin-induced hepatotoxicity has been associated with elevated AST and total bilirubin levels in other test animals [2, 3, 14, 15]. No clinical signs consistent with maternal intoxication (reduced feed intake and weight gain, lethargy, death) resulted from fumonisin administration. Reduced fetal body weight, delayed ossification and delayed organ development have been reported to occur in fetuses as a result of maternal intoxication from exposure to a variety of compounds [16]. In the present study, average fetal weights for treated females were similar to those for control females (Table 4). Internal organs and skeletons were all of the appropriate stage of development for term fetuses [17]. Therefore, fetal losses are believed to represent the combined direct effects of FB_1 and FB_2 on fetal development rather than secondarily to maternal intoxication.

The number of litters with fetal losses increased significantly as the dose of FB_1 increased. Increases in fetal resorptions and deaths were similar to earlier results [8], however, larger daily doses (18EX vs 12EX) were required to induce total litter losses in the present study because treatments were restricted to two days rather than four. Fumonisin B_1 has been shown to inhibit sphingolipid synthesis in vitro [18] and in vivo [19] and to increase the tissue content of free sphinganine and free sphingosine [19]. The effect this has on developing fetuses is unclear, although sphingosine and other sphingolipid breakdown products are reported to have a variety of biological and pathological affects on cell function [20]. The pattern of increasing fetal losses is consistent with compounds that interfere with cellular replication in embryonic tissues causing generalized tissue and prenatal death with increasing

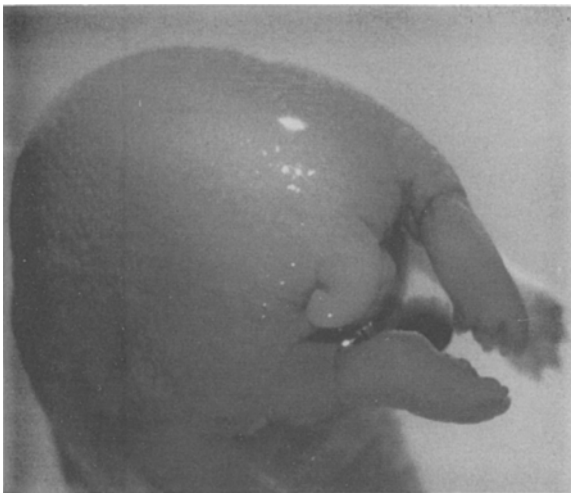

Fig. 1. Example of 15 day fetus with curled tail after prenatal fumonisin exposure. Dam had received 12 mg fumonsin B_1/kg in culture extract on days 8 and 9 of gestation. (Photo magnification: ×12)

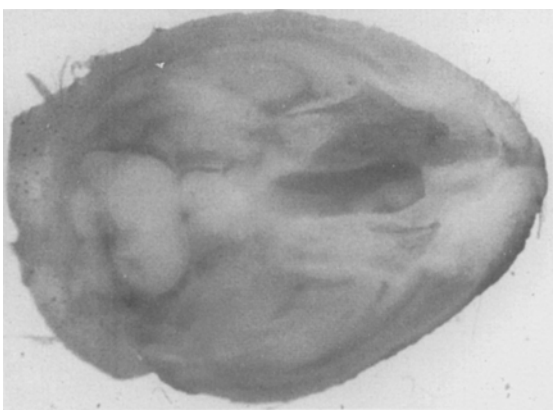

Fig. 2. Example of 15 day fetus with cleft palate after prenatal fumonisin exposure. Dam had received 18 mg fumonisin B_1/kg in culture extract on days 8 and 9 of gestation. (Photo magnification: ×6)

dose [21]. Nonspecific developmental toxicity (fetal death and resorption) can also occur secondary to compromised placental function [19]. Which fetal tissue or tissues are being adversely affected by fumonisin is unknown.

Fumonisin B_2 has been shown to have similar toxicological and cancer initiating activity to FB_1 in rats [22]. Although not statististically significant, the added effect of FB_2 in culture extract administered to 18EX females may account for the slightly increased numbers of fetal resorptions and deaths per litter compared to 18P females.

Congenital malformations may be hereditary, occur spontaneously, or result from exposure to exogenous compounds during prenatal development. The spontaneous occurrence rate of kinky tails, digit reduction, and cleft palates is reported to range from 0.05 to 0.6, 0.05 to 0.4, and 0.2 to 2.7 per 1000 neonates, respectively, in mice, rats and rabbits [23]. In hamsters, 0.5% of control fetuses were reported to be malformed [17]. The incidence of malformations, especially curled tails, increased with increasing dose of fumonisin. Examination of stained skeletons from affected litters failed to demonstrate additional spinal abnormalities (i.e. spina bifida) which often accompany tail defects [23]. Although malformations increased, they were not statistically significant because affected fetuses were clustered into a few litters. Factors affecting this biological variability are not known. Also, at higher doses (18EX) resorption of entire litters reduced the possibility of malformations being observed.

Further investigation of the developmental toxicity of FB_1 is required to identify its mode of action on fetal development, whether acting directly on fetal tissues or by interrupting normal fetomaternal communication. Measuring the sphinganine to shingosine ratio in dams and their offspring may serve to identify the target tissues of fumonisin. Abnormal sphinganine to shingosine ratios in fetuses in the presence of normal maternal ratios may indicate the unique susceptibility of the conceptus to fumonisins, whereas, normal ratios in fetuses would indicate an indirect action of fumonisin on maternal and/or placental tissues [19].

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