Bull. Environ. Contam. Toxicol. (1996) 57:354-360

Bulletin of Environmental Contamination and Toxicology

Developmental Toxic Effects of Fusaric Acid in CD1 Mice

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Received: 25 October 1995/Accepted: 18 March 1996

Fusaric acid (FA) is a toxic metabolite produced by *Fusarium moniliforme* in corn and other cereal grains including barley, wheat, millets and sorghum (Christensen and Kaufmann, 1969; Joffe, 1984; Ueno and Ueno, 1978). Smith and Sousadias (1993) demonstrated FA contamination as high as 35.76 mg/kg of swine feed and 135 mg/kg in high moisture corn occurring simultaneously with other mycotoxins such as vomitoxin and zearalenone. Human per capita consumption of corn and corn products in the United States is about 75 lb/yr (USDA, 1994). Although corn destined for human consumption is not specifically screened for FA contamination, since 79 to 100% of grain and animal feed samples on farms contained FA (Smith and Sousadias, 1993) the likelihood of exposure to FA in humans, including pregnant women, exists. Fusaric acid is reported to be a potent inhibitor of dopamine β-hydroxylase (DBH) (Suda *et al*, 1969) and has caused vomiting and neurological disorders in pigs (Smith and MacDonald, 1991). Since DBH inhibition is likely to lead to reduced catecholamine synthesis and since catecholamines appear to be involved in normal development of the secondary palate (Greene and Garbarino, 1984) it is likely that exposure to FA in pregnant animals could lead to cleft palate in their offspring via reduced catecholaminergic activity. Matsuzaki *et a1* (1976 a,b) reported no adverse effects in dams and only minor skeletal ossification defects in the offspring of DDB4 mice treated with as high as 125 mg FA/kg. However, these studies involved exposure of FA only on gestation days (GD) 7 through 13. In mice, period of palate development extends through GD 14.5 and exposure through GD 15 is necessary to evaluate FA's cleft palatogenic potential.

Several research groups proposed and provided supportive evidence to the theory that many structurally diverse chemicals including secalonic acid D (SAD), and the adrenergic agent orciprenaline sulfate may cause cleft palate by elevating maternal plasma corticosterone (MPC) levels (Eldeib and Reddy, 1990; Hansen *et al*, 1988; Jida *et al* 1988). The facts that the glucocorticoids themselves are cleft palatogenic (Baxter and Fraser, 1950) and that the administration of dimethyl sulfoxide (DMSO) abolished SAD-induced MPC elevations and also drastically reduced the incidence of

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cleft palate in the offspring of SAD-exposed mice (Eldeib and Reddy, 1990) support the MPC theory. These studies were designed to evaluate the developmental toxic effects, especially the cleft palatogenic effects, of FA exposure in CD-1 mice at a more relevant gestational period than previously investigated and their association with MPC elevations in FA-treated dams.

MATERIALS AND METHODS

Mature (> 9 weeks) female and male CD-1 mice from Charles River (Wilmington, MA) were cohabited overnight in a 2:1 ratio in polypropylene cages containing aspen wood bedding (Northeastern products, Warrensburg, NY) in rooms maintained under controlled environmental conditions (72±2 °F, R.H. 50% and 12 hr light/dark cycle). Females with vaginal plugs on the next morning were presumed to be at GD zero and were randomly assigned to treatment groups with ad libitum access to feed (Purina rat chow) and water.

Fusaric acid (purity > 99%) was obtained from Sigma Chemical Co. (St. Louis, MO) and doses (established from our preliminary study) equivalent to 0, 75, 87.5, 100, 112.5, 125, 137.5 and 150 mg/kg body weight dissolved in sterile distilled water were administered by gavage daily at a rate of 0.1 mL/40g body weight on GD 7 through 15. On GD 12, blood (100 µL) was collected from the orbital sinus of each mouse at 9:00 am±1 hr to measure corticosterone. The choice of GD 12 was based on the facts that this time point falls within the critical window of palate development and that several studies (see introduction) demonstrated corticosterone elevations in treated dams at this time point.

Corticosterone levels in plasma were assayed by radioimmunoassay (ICN Biomedicals, Costa Mesa, CA) with a detection limit of 25 ng/mL. Separation of antibody bound and free corticosterone was achieved by precipitating the bound steroid using a second antibody (goat anti-rabbit gamma globulin). Diluted (1:200 with steroid diluent) unextracted samples (100 µL) were assayed in triplicate, and the results were expressed as ng/mL. The standard curve after logit transformation (Robard and Frazier, 1975) was used for the interpolation of sample values using an IBM computer-assisted program.

On GD 18, the mice were weighed and euthanized by cervical dislocation, the uterus was opened, and number of implantation sites, resorptions, stillborn and live offspring were recorded. Total litter weight was calculated (by adding weights of all live pups) as a true reflection of the combined embryocidal and fetotoxic effects of FA. Maternal weight gains were calculated as the difference in the weight of the dam between GD 7 and 18. Following gross examination, half the number of fetuses were fixed in Bouin's fluid for soft tissue examination and the remaining were cleared in KOH and stained with alizarin red S for skeletal examination (Wilson, 1965). Maternal liver, kidneys, adrenals and brain were stored in Z-fix (Anatech Ltd, Battle Creek, MI) for examination by light microscopy following staining of the tissues with

hematoxylin and eosin.

Number of observations equaled the number of dams treated with FA or vehicle. Analysis of variance was performed to compare the dose response of FA on maternal weight gains, litter weights, numbers of implants and live offspring, and weights of live offspring. Data on the number of resorptions were arcsine-transformed (Mosteller and Youtz, 1961) prior to ANOVA. Arcsine transformation is often used prior to statistical analysis to improve normality of distribution and to stabilize variance of binomial data such as that of malformations and resorptions (Manson and Kang, 1994). Least significant difference (LSD) test was used to discern significant ($P \leq .05$) effects of various doses of FA.

RESULTS AND DISCUSSION

Fusaric acid was lethal to mice at doses of ≤ 112.5 mg/kg with mortality reaching 100% at 137.5 mg/kg (Table 1). Maternal body weight gains between GD 7 and 18 were significantly reduced at 125 mg FA/kg (Table 1). The number of resorptions was increased and the number of live offspring and their body weight decreased significantly at doses of 112.5 mg FA/kg body weight or higher (Table 1). This effect likely contributed to the decrease in both maternal weight gains and total litter weight (Table 1). The average offspring body weight was significantly lower in dams treated with doses of FA greater than 100 mg/kg (Table 1). Matsuzaki *et al* (1976 a,b) failed to observe maternal mortality even at a dose of 125 mg/kg. Maternal mortality at lower doses in our study may at least partly be explained by longer duration of FA administration (GD 7-15) in our study compared to that (GD 7-13) of Matsuzaki *et al* (1976, a, b). Significant reduction in the body weight of dams treated with 125 mg/kg was shown by Matsuzaki *et al.* (1976 b) but only on GD 14, one day after the last dose. These differences disappeared by GD 18. In our study, longer exposure, as can be expected, resulted in weight gain reduction that could not be compensated following cessation of exposure.

No gross or visceral malformations (including cleft palate) were noticed in the offspring of dams treated with FA at any of the doses used in the study. Phalangeal ossification was deficient in two offspring of only one dam treated with 100 mg FA/kg body weight. Whereas 5 control offspring exhibited extra ribs (not shown). A lack of dose response in these effects indicates this to be a random effect in our studies unrelated to FA-treatment. Matsuzaki *et al* (1976 a) also observed an increased incidence of non-ossified occipital bone and sternebrae along with ossification deficits of the extremities at 125 mg FA/kg bodyweight. The lack of ossification deficits in our study following prolonged exposure is unexpected and can only be attributed to strain differences (DDBH vs CD1) in susceptibility to FA. Histological examination of maternal liver, brain, kidneys and adrenals revealed no changes attributed to the treatment with FA at any dose at the light microscopic level compared to controls.

Table 1. Effect of Fusaric Acid on Pregnant CD1 Mice and the Development of Their Offspring[a].

Dose (mg/kg)	No. of Dams (% mortality)	Maternal Weight Gain (g)	Implants	Percent (of implants)		Offspring Body Weight (g)	
				Resorptions & Fetal Deaths	Live Offspring	Average	Total Litter
0.0	10(0)	22.9 ±1.1	13.1 ±0.64	4.3 ±2.0	95.7 ±2.0	1.38 ±0.02	17.2 ±0.73
75.0	10(10)	24.2 ±1.0	14.1 ±0.81	5.8 ±2.5	94.2 ±2.5	1.26 ±0.06	16.5 ±0.90
87.5	11(0)	17.9 ±2.3	12.8 ±0.97	6.0 ±2.1	94.0 ±2.1	1.20 ±0.05	14.7 ±1.4
100.0	10(10)	18.6 ±2.7	13.0 ±1.1	9.5 ±2.6	90.5 ±2.6	1.21 ±0.04	14.3 ±1.28
112.5	11(36.4)	14.7 ±6.1	11.3 ±1.8	44.8[b] ±19.5	55.2[b] ±19.5	1.17[b] ±0.07	13.6 ±1.36
125.0	10(50)	8.5[b] ±7.7	10.0 ±0.0	62.0[b] ±23.3	38.0[b] ±23.3	1.15[b] ±0.02	10.7[b] ±0.48

[a] Fusaric acid was administered by gavage from gestation day 7 through 15. Mortality was 100% at doses of 137.5 mg/Kg or higher.

[b] Means (±SE) bearing this superscript are significantly ($P \leq 0.05$) different from controls in the same column.

Table 2. Effects of Fusaric Acid on Maternal Plasma Corticosterone[a] Elevations and on Palate Development in Fetal Mice Compared with Selected Previously Published Data.

Treatment [Dose (mg/kg); GD and route of exposure)	Plasma Corticosterone (% of control)	Cleft Palate in % of live offspring
Control	100	0
Fusaric Acid[b] (Upto 100 mg/kg, GD 7-15, oral)	95	0
Orciprenaline Sulfate[c] (500 mg/kg, GD 11-13, oral)	290	12
Phenytoin[d] (75 mg/kg, GD 10, intraperitoneal)	640	96
Secalonic Acid D[e] (35 mg/kg, GD 11, intraperitoneal)	421	57

[a] Plasma corticosterone levels were assayed on GD 12 and/or 24 hr after administration of the teratogen.
[b] Corticosterone levels for all doses of FA were pooled because of lack of differences ($P \leq 0.05$) from those of controls.
[c] Data from Iida et al (1988) Tables 8 and 9.
[d] Data from Hansen et al (1988). From Table 1 and weighted averages of percentages from two replicates in Table 2.
[e] Data from Eldeib and Reddy (1990).

The examination of the data from the present studies suggest that the multiple dose no-observed-adverse-effect-level (NOAEL) for developmental toxic effects of FA may be between 100 and 112.5 mg FA/kg body weight. This NOAEL is lower than that gleaned from previous studies (Matsuzaki et at., 1976 a, b). The impact of embryotoxic effects of FA either alone or in combination with other toxins of Fusaria (Smith and Sousadias, 1993) in domestic animals can be significant. The significance of ossification deficits in the offspring, however, seems minimal since Matsuzaki *et al,* (1976 b) demonstrated no lasting post-natal effects in these offspring.

Maternal plasma corticosterone levels in the control mice on GD 12 averaged 461±40 ng/mL. In each of the treated groups, the MPC values were similar to controls (not shown) and averaged 440±51 ng/mL when pooled (Table 2).

The results of our studies with the mycotoxin secalonic acid D and the xanthine

alkaloid, caffeine (Eldeib and Reddy, 1990; Reddy *et al*, 1994) as well as studies from other laboratories with phenytoin (Hansen *et al*, 1988) and orciprenaline sulfate (Iida *et al*, 1988) supported the concept (see introduction) that toxin-induced elevations in MPC levels may be causally involved in the pathogenesis of cleft palate in the offspring (Table 2). If this hypothesis were to hold true, those agents incapable of inducing cleft palate in the offspring should be incapable of inducing MPC elevations. Results of this study with FA are consistent with this reasoning.

Our studies demonstrated the embryotoxicity of FA at doses lower than those previously shown and have provided evidence consistent with the hypothesis that agents capable of inducing cleft palate in the offspring of exposed mice may do so at least partly, by elevation of MPC.

Acknowledgements. These studies were partially supported by a Summer Research Fellowship (CAL), Phi-Zeta research grant and Committee on Research grant from the College of Veterinary Medicine, University of Missouri, Columbia. Authors extend thanks to Ms. Jennifer Winn for her expert typing of the manuscript.

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