Effects of Rubratoxin B on Prenatal Development in Mice

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Introduction

Rubratoxin B is one of a number of diverse metabolites produced by storage molds that are toxic either by contact or by inadvertent ingestation of the toxin when present in foods or feeds. The possibility exists that prenatal mortality and malformation in humans or in domestic animals may be caused by consumption of agricultural commodities contaminated with mycotoxins such as rubratoxin.

Rubratoxin B is produced by isolates of Penicillium rubrum and P. purpurogenum. Although both species have been isolated from a number of field outbreaks, no information is available concerning the natural distribution of this metabolite in human foodstuffs or animal feeds. In addition, information on the effects of purified rubratoxin under controlled environmental conditions is limited. HAYES and WILSON (1970) reported its effect on liver composition and metabolism in mice but offered no explanation as to its mechanism of toxicity. The acute and chronic toxicity of rubratoxin to a number of species including mice has been reported by WOGAN et al. (1971). These workers also have evidence of the potentiation of the lethal action of rubratoxin B, but not of the carcinogenic action of aflatoxin B_1, in rats treated simultaneously with both mycotoxins. Radioactivity derived from rubratoxin B has been reported in liver and kidneys of rats and mice 30 minutes after administration (HAYES, 1972).

No demonstration of the effect of rubratoxin on mammalian embryos has been reported. However, other mycotoxins are known embryocides and teratogens. LE BRETON et al. (1964) reported that in rats a dose of 300 ug aflatoxin B_1 caused fetal death with hemorrhages at the uteroplacental junction. BUTLER and WIGGLESWORTH

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(1966) reported a slight reduction in placental weight at term when one quarter of the LD_{50} for non-pregnant female rats was given. ELIS and DI PAOLO (1967) have described the development of central nervous system defects when 4 mg/kg aflatoxin was given upon day 8 of pregnancy to golden hamsters. The only other mycotoxin to have received recognition for its effects on the embryo is ochratoxin. Ochratoxin A (6.25 mg/kg) induced fetal death and resorption in rats, but its hydrolysis product, dihydroisocoumarin, had no effect (STILL et al., 1971). Ochratoxin A (3 mg/kg) has also been found to be highly teratogenic in mice (HOOD et al., 1972).

Since information on teratogenic effects of mycotoxins is limited, the present work with rubratoxin B was undertaken to determine if this mold metabolite was a teratogen and if its effects were similar to those of other mycotoxins previously investigated.

Materials and Methods

Sexually mature nulliparous female Swiss-Webster mice (30-35 g) of the CD-1 strain (Charles River Mouse Farms) were caged with males of the same strain. Observation of copulatory plugs defined day one of gestation. Pregnant females were given food and water *ad libitum*.

Treatment consisted of a single ip injection of rubratoxin on one of days 6-12 of gestation. Rubratoxin was isolated from cultures of *P. rubrum* and purified by the method of HAYES and WILSON (1968). Dose levels were 0.4, 0.6, 0.9 or 1.2 mg/kg body weight. The mycotoxin was dissolved in propylene glycol to which an equal amount of water was added. Control animals were injected with solvent alone equivalent to the volume of the highest dose (1.2 mg/kg) of rubratoxin. Untreated controls also were used.

Mice were killed on day 18 by an overdose of ether. The uterus was examined and the position of each fetus (live or dead) and the numbers of fetuses and resorption sites were recorded. Fetuses were removed from the uterus, stripped of fetal membranes, blotted to remove excess fluid and individually weighed. Fetuses were then examined under magnification for external malformations. Fetal weight, malformation and mortality data were subjected to analysis of variance for evaluation

of the effects of dose and treatment day. Mean differences were determined by the method of Newman-Keuls (WINER, 1971).

Results and Discussion

Single ip injections of rubratoxin resulted in significant ($P < 0.01$) increases in embryonic mortality on all days and at all dose levels tested when compared with the results from mice injected with solvent or untreated controls (Tables 1 and 2). Occurrence of mortality approximated time of injection as evidenced by the size of the resorption sites observed by day 18. Embryonic deaths were correlated with dose level and were day-dependent. Treatment on day 8 resulted in 100% mortality at dose levels above 0.4 mg/kg. On all other days there were some survivors, although some litters were totally resorbed on each day.

Propylene glycol did not cause a significant increase in embryonic mortality. However, the percentage of resorptions and deaths on days 8, 9 and 11 in mice treated with the solvent was 2 to 3 times that for untreated litters. Lack of statistical significance for these differences may be due to the variable nature of the data from these solvent-treated groups, particularly because of the completely resorbed litter associated with the day 8 and 9 groups and two day 11 litters with large numbers of resorptions. These litters considerably increased the mortality rates for the three groups but at the same time greatly increased the variability associated with the data.

Rubratoxin treatment also caused a significant ($P < 0.01$) decrease in fetal weights when administered on all days and dose level combinations with surviving fetuses except the day 12, 0.6 mg/kg combination ($P < 0.05$) and the day 9, 0.6 and 0.9 mg/kg combinations (N.S.). Rubratoxin treatment resulted in an average fetal weight by day 18 of pregnancy of as little as one-half that of untreated or solvent-treated fetuses in some cases (e.g., day 6, 0.9 mg/kg and day 7, 1.2 mg/kg). Fetuses from solvent-injected mothers did not differ significantly from those from untreated mothers with regard to weight with the exception of those treated on day 7 (Tables 1 and 2).

TABLE 1

Effect of Rubratoxin B on Development in Mice.

Day	Treatment[a]	Dose (mg/kg)	No. Pregnant Mice	Total Implantations	Litters Totally Resorbed	Fetal Weights (g ± s.e.)	% Resorbed or Dead	% Grossly Malformed[b]
6	R	0.6	6	77	3	0.70±0.02[df]	53.25[df]	5.56
		0.9	7	72	3	0.50±0.03[df]	79.17[df]	20.00
		1.2	6	53	6		100.00[df]	
	PG		5	55	0	0.92±0.01	7.27	
7	R	0.6	6	69	2	0.63±0.02[df]	50.72[df]	12.12
		0.9	6	71	5	0.64±0.04[df]	84.51[df]	45.45
		1.2	5	57	4	0.46±0.03[df]	89.47[df]	83.33
	PG		6	72	0	0.86±0.02[e]	6.94	
8	R	0.4	5	55	2	0.69±0.03[df]	49.09[df]	85.71
		0.6	6	77	6		100.00[df]	
		0.9	6	74	6		100.00[df]	
		1.2	6	71	6		100.00[df]	
	PG		7	77	1	0.97±0.02	14.29	

[a] R = rubratoxin in 1:1 propylene glycol:H_2O; PG = 1:1 propylene glycol:H_2O with a volume equivalent to that used as the solvent for the 1.2 mg/kg rubratoxin dose.

[b] As % of intact fetuses.

[d] Significantly different from the solvent injected controls: ($P < 0.01$).

[e,f] Significantly different from the untreated controls: [e]($P < 0.05$), [f]($P < 0.01$).

TABLE 2

Effects of Rubratoxin B on Development in Mice.

Treatment[a] Day	Dose (mg/kg)	No. Pregnant Mice	Total Implantations	Litters Totally Resorbed	Fetal Weights (g ± s.e.)	% Resorbed or Dead	% Grossly Malformed[b]
9	R 0.6	5	56	3	0.89±0.02	78.57[df]	8.33
	0.9	5	61	4	0.95±0.05	88.52[df]	
	1.2	6	75	6		100.00[df]	
	PG	7	71	1	0.96±0.01	18.31	
10	R 0.6	5	61	4	0.82±0.03[df]	78.69[df]	
	0.9	5	50	5		100.00[df]	
	1.2	5	68	4	0.61±0.01[df]	97.06[df]	
	PG	5	61	0	1.01±0.02	4.92	
11	R 0.6	5	61	0	0.77±0.01[df]	52.46[df]	3.23
	0.9	5	53	5		100.00[df]	
	1.2	6	72	6		100.00[df]	
	PG	6	66	0	1.00±0.02	13.64	
12	R 0.6	5	58	0	0.88±0.02[ce]	32.76[df]	
	0.9	5	59	1	0.80±0.03[df]	52.54[df]	
	1.2	5	51	3	0.76±0.02[df]	74.51[df]	
	PG	5	60	0	0.96±0.02	8.33	
	Untreated	11	106	0	0.96±0.01	6.09	

[a] R = rubratoxin in 1:1 propylene glycol:H_2O; PG = 1:1 propylene glycol:H_2O with a volume equivalent to that used as the solvent for the 1.2 mg/kg rubratoxin dose.

[b] As % of intact fetuses.

[c,d] Significantly different from the solvent injected controls: [c]($P < 0.05$), [d]($P < 0.01$).

[e,f] Significantly different from the untreated controls: [e]($P < 0.05$), [f]($P < 0.01$).

The effective dose levels employed in this investigation were considerably lower than the lethal dose for adult mice (MLD $\cong$ 2.0 mg/kg, $LD_{50} \cong$ 2.6 mg/kg). Rubratoxin differs in this respect from many teratogens which are effective only in doses close to the lethal dose for adults.

Some of the surviving fetuses developed abnormalities after rubratoxin treatment (Tables 1, 2 and 3). Most developmental anomalies occurred in fetuses whose mothers were treated on days 6, 7 and 8 with a few defective fetuses seen on days 9 and 11. No abnormalities were found in solvent-control or untreated fetuses. There were no survivors of the treatments initially administered on gestation day 8. Treatment on day 8 with a lower dose (0.4 mg/kg) allowed some fetuses to survive and thus reveal teratogenic effects. Gestation day 8 appears to be a critical time with regard to developmental effects of rubratoxin with 51% of fetuses surviving and 86% of those malformed.

The most common defects noted were exencephalies, malformed pinnae, malformed jaws, umbilical hernias and open eyes. The anomalies produced by rubratoxin were typical of defects induced by "general teratogens" which produce a variety of effects in mice particularly involving the central nervous system, eyes and tail. More specific teratogens tend to produce specific deformities which involve a relatively more narrow range of tissues and structures (e.g., cleft palate due to cortisone treatment).

The results of the present study clearly demonstrate that single doses of rubratoxin B that were not lethal to pregnant females produced growth and developmental retardation, death, and congenital malformations in fetuses of mice. Differential effects of this mycotoxin were dependent on dose of the agent and on the stage of embryonic development.

The ability of rubratoxin to cause embryonic deaths and malformations at dosages considerably below levels lethal to adults indicates that this mycotoxin is a potent embryocide. Consumption of foods contaminated by P. rubrum or by P. purpurogenum could result in abortion or deformed offspring. Although it is impossible to extrapolate directly from rodents to

TABLE 3

Developmental Anomalies Induced by Rubratoxin B in Mice. Day and Dose Level vs Response[a].

Anomaly	Gestation day administered/Dose level[b]													
	6/0.6		6/0.9		7/0.6		7/0.9		7/1.2		8/0.4		9/0.6	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Exencephaly							3/11	27.27			9/28	32.14		
Open Eye					1/33	3.03	2/11	18.18	3/6	50.00	5/28	17.86		
Exophthalmia													1/12	8.33
Anophthalmia									1/6	16.67	2/28	7.14		
Missing Pinna							1/11	9.09			1/28	3.57		
Displaced Pinna							1/11	9.09						
Malformed Pinna											15/28	53.57		
Malformed Jaws									3/6	50.00	10/28	35.71		
Cleft Palate-lip									1/6	16.67				
Digital Malformation											1/28	3.57		
Ectrodactyly											2/28	7.14		
Micromelia									1/6	16.67				
Umbilical Hernia	2/36	5.56	3/15	20.00					2/6	33.33			1/12	8.33
Eventration			1/15	6.66	3/33	9.09								
Short Tail									1/6	16.67				

[a]Solvent treated (1:1 propylene glycol:H_2O) and untreated control fetuses exhibited no gross abnormalities.

[b]N = No. abnormal/total fetuses observed.

humans, the teratogenic effect of rubratoxin and its previously reported hepatotoxicity indicate that this mycotoxin should be treated with caution.

Summary

Single doses (0.4, 0.6, 0.9, or 1.2 mg/kg) of rubratoxin B were given ip to albino mice on days 6-12 of gestation and fetuses were examined on day 18. Results indicated that rubratoxin is a growth retardant, a potent embryocide and a teratogen. Exencephaly, malformed pinnae, malformed jaws, umbilical hernia and open eye were the most commonly noted developmental anomalies associated with rubratoxin treatment.

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