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Susceptibility of Mice to Phenytoin-Induced Cleft Palate Correlated with Inhibition of Fetal Palatal RNA and Protein Synthesis (41255)

BABASAHEB R. SONAWANE* AND ALLEN S. GOLDMAN†

*Divisions of *Clinical Pharmacology and †Human Genetics and Teratology, The Children's Hospital of Philadelphia and Department of Pediatrics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104*

Abstract. Phenytoin administered to pregnant mice during the critical embryonic period of palatal differentiation produced 50% cleft palates in the Ajax (A/J) strain compared to 1.6% clefts in the C57BL/6 (B6) strain of mice. Furthermore, a single maternal injection of phenytoin produced a significantly greater and more persistent decrease in fetal palatal RNA and protein synthesis in the sensitive A/J strain compared to that in the insensitive B6 strain of mice. Thus, these differential effects of phenytoin on RNA and protein synthesis are associated with the differential susceptibility to the teratogenic action of phenytoin in the two strains.

Phenytoin has been shown to be teratogenic to both experimental laboratory rodents and humans (1, 2). In humans, phenytoin produces cleft lip and/or cleft palate (3–6). Phenytoin can cross the placenta readily (7–9) and is known to induce high frequencies of cleft palate in several strains of mice (10–12). Recently, Walker (13) demonstrated that phenytoin induced general developmental retardation and diminution of embryonic muscular movements at an early embryonic stage indicating interference with palatal shelf rotation as observed with cortisone and triamcinolone (14).

Both glucocorticoids (15, 16) and phenytoin (17) produce cleft palate in susceptible mouse strains containing the *H-2*^{a} histocompatibility complex on chromosome 17 (A/J and B10.A). Zimmerman *et al.* (18) have shown that glucocorticoid exposure during the critical embryonic period causes inhibition of RNA and protein synthesis in mouse fetal palates. Thus, we have investigated whether phenytoin may also cause inhibition of RNA and protein synthesis in fetal palates during the critical period. In the present studies, we report that phenytoin significantly and persistently depresses fetal palatal RNA and protein synthesis in the cleft-palate-sensitive Ajax (A/J) strain compared to that in the insensitive C57BL/6 (B6) strain of mice.

Materials and Methods. The mouse strains used were Ajax (A/J) and C57BL/6 (B6) (Jackson Laboratory, Bar Harbor, Maine). Mice were mated overnight and a vaginal plug was considered as Day 0 of gestation. Phenytoin (Dilantin—phenytoin sodium injection, Parke Davis and Company, Detroit, Mich.), 50 mg/kg body wt, was injected subcutaneously on the 12th day of gestation. The effect of phenytoin on the rate of RNA and protein synthesis in fetal palate, body (excluding palate), and maternal liver was measured by the incorporation of [^{3}H]uridine and [^{14}C]leucine into RNA and protein, respectively, as described by Zimmerman *et al.* (18). Pregnant mice were sacrificed at the indicated time periods (3, 24, 48, and 72 hr), 2 hr after intraperitoneal (ip) injection of 10 μCi of [^{3}H]uridine (sp act 8 Ci/mmole) and 0.5 hr after ip injection of 10 μCi of [^{14}C]leucine (sp act 51.63 mCi/mmole). Upon sacrifice, uterine horns were removed and placed on ice. Each embryo was separated from its placenta and membranes with the aid of a dissecting microscope. The fetal palates were dissected, washed in physiological saline, blotted, and pooled in each litter, and then flash-frozen at −80°. Tissues were homogenized in cold distilled water (5% W/V) using a motor-driven Thomas homogenizer. Triplicate aliquots of 1.0 ml of each were removed and made to 5% with cold trichloroacetic acid (TCA). The samples were centrifuged at 20000 *g* for 10 min and

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TABLE I. STRAIN DIFFERENCES IN PHENYTOIN-INDUCED CLEFT PALATE FORMATION IN MICE[a]

Strain	No. of litters	Resorptions	Percentage resorptions	Fetuses with cleft palate	Percentage of fetuses with cleft palate
Ajax	6 (36)	12	33.3	18	50*
C57BL/6	9 (63)	1	1.6	1	1.6

[a] Phenytoin, sodium (50 mg/kg body wt) was injected (sc) on gestation Days 11–14 and fetuses were dissected by caesarean section on Day 19 of pregnancy.
* Significantly different from C57BL/6 strain of mice, $P < 0.005$.

pellets were washed twice with 5 ml cold 5% TCA. The final washed pellet was dissolved overnight in NCS tissue solubilizer (Amersham–Searle) at 37° in a shaking water bath. Hydrogen peroxide was added to each tube to decolorize samples if necessary. ^{3}H- and ^{14}C-amino acid incorporation in tissues was measured simultaneously in 10 ml of Aquasol (New England Nuclear, Boston, Mass.) using a Packard Tricarb Liquid Scintillation Spectrophotometer. Radioactivity measurements were made with appropriate quench correction with automatic external standardizations. The incorporation of labeled amino acids was calculated as disintegrations per minute per milligram of tissue.

Results. *Cleft palate formation.* Phenytoin resulted in 33% resorptions in the A/J strain, while the B6 strain of mice had less than 2% resorptions (Table I). Phenytoin produced 50% clefts in A/J compared to only 1.6% clefts in B6 mice.

Fetal palatal RNA synthesis. In A/J mice fetal palatal RNA synthesis was significantly inhibited (88%) 3 hr after drug administration, but in the B6 strain the degree of inhibition was less (41.5%) (Table II). The significant inhibition of fetal palatal RNA synthesis continued up to 48 hr in A/J mice, however, phenytoin did not inhibit RNA synthesis in B6 fetal palates after 3 hr (Table II).

Fetal palatal protein synthesis. Protein synthesis in A/J fetal palates was also inhibited, but not to the same extent as RNA synthesis (Table III). The maximum inhibition of protein synthesis occurred at 48 hr after drug administration and thereafter returned to control levels. By contrast, fetal palatal protein synthesis was unaffected by phenytoin administration in B6 mice (Table III).

RNA and protein synthesis in fetal body. In A/J fetal bodies (excluding palates) RNA synthesis was inhibited at 3, 24, and 48 hr

TABLE II. EFFECT OF PHENYTOIN ON RNA SYNTHESIS IN MOUSE FETAL PALATE[a]

Strain	Time (hr): Treatment	[^{3}H]Uridine incorporated/mg tissue (wet wt)[b] 3	24	48	72
A/J	Control	202.8 ± 24.9(4)	90.9 ± 29.9(7)	85.8 ± 25.3(6)	46.6 ± 3.8(7)
	phenytoin	43.8 ± 3.4(4)	57.8 ± 18.6(6)	26.5 ± 7.8(6)	59.4 ± 24.6(10)
	% inhibition	88.4	36.4	69.2	—
	P =	3.5×10^{-6}	0.039	0.0003	N.S.
B/6	Control	106.0 ± 7.1(4)	92.5 ± 24.9(8)	129.8 ± 21.5(6)	84.4 ± 16.2(6)
	Phenytoin	62.0 ± 17.5(4)	87.7 ± 23.5(8)	136.1 ± 14.8(6)	92.8 ± 8.6(6)
	% inhibition	41.5	—	—	—
	P =	0.034	N.S.	N.S.	N.S.

[a] Phenytoin (50 mg/kg) or vehicle to control animals was administered (sc) on the 12th day of gestation to A/J and B/6 pregnant mice. RNA synthesis was measured at indicated time periods as described under Materials and Methods.
[b] Values represent mean dpm ± SE; parentheses indicate numbers of individual litters examined.

TABLE III. EFFECT OF PHENYTOIN ON PROTEIN SYNTHESIS IN MOUSE FETAL PALATE[a]

Strain	Time (hr): Treatment	[^{14}C]Leucine incorporated/mg tissue (wet wt)[b] 3	24	48	72
A/J	Control	83.4 ± 14.8(5)	114.1 ± 6.6(8)	138.7 ± 9.7(6)	103.9 ± 10.6(6)
	Phenytoin	53.6 ± 9.9(4)	80.0 ± 6.9(8)	60.9 ± 9.0(6)	118.6 ± 25.2(10)
	% inhibition	34.7	29.9	56.1	—
	P =	0.01	0.032	0.00015	N.S.
B/6	Control	179.0 ± 47.0(4)	124.9 ± 9.3(8)	172.6 ± 10.1(6)	134.5 ± 14.3(6)
	Phenytoin	145.3 ± 28.8(4)	109.5 ± 12.2(8)	153.9 ± 29.2(6)	138.7 ± 11.5(6)
	% inhibition	—	—	—	—
	P =	N.S.	N.S.	N.S.	N.S.

[a] Phenytoin was administered as described in footnote *a*, Table I. Protein synthesis was measured by indicated time periods as described under Materials and Methods.
[b] Values represent mean dpm ± SE; parentheses indicate numbers of individual litters examined.

TABLE IV. EFFECT OF PHENYTOIN ON RNA SYNTHESIS IN THE MOUSE FETAL BODIES[a]

Strain	Time (hr): Treatment	[^{3}H]Uridine incorporated/mg tissue (wet wt)[b] 3	24	48	72
A/J	Control	196.5 ± 26.8(6)	98.8 ± 22.5(7)	94.5 ± 18.0(9)	56.9 ± 3.3(12)
	Phenytoin	51.5 ± 8.8(12)	43.1 ± 13.8(5)	35.3 ± 11.1(6)	67.5 ± 19.9(18)
	% inhibition	73.8	56.4	62.4	—
	P =	7.2×10^{-6}	0.043	0.014	N.S.
B/6	Control	99.7 ± 9.2(4)	114.9 ± 18.4(8)	133.8 ± 16.5(6)	112.6 ± 16.3(6)
	Phenytoin	53.5 ± 12.8(4)	112.5 ± 17.9(8)	146.0 ± 19.4(6)	134.0 ± 10.5(6)
	% inhibition	46.3	—	—	—
	P =	0.013	N.S.	N.S.	N.S.

[a] Phenytoin was administered as described in footnote *a*, Table 1. RNA synthesis was measured in the mouse fetal bodies excluding palates as described under Materials and Methods.
[b] Values represent mean dpm ± SE; parentheses indicate numbers of individual litters examined.

TABLE V. EFFECT OF PHENYTOIN ON PROTEIN SYNTHESIS IN MOUSE FETAL BODIES[a]

Strain	Time (hr): Treatment	[^{14}C]Leucine incorporated/mg tissue (wet wt)[b] 3	24	48	72
A/J	Control	120.2 ± 18.1(6)	121.4 ± 18.9(7)	143.6 ± 12.1(9)	110.1 ± 11.0(12)
	Phenytoin	100.7 ± 13.3(12)	101.9 ± 10.4(5)	78.4 ± 15.6(6)	124.6 ± 26.3(18)
	% inhibition	—	—	—	—
	P =	N.S.	N.S.	0.003	N.S.
B/6	Control	134.8 ± 29.6(4)	148.1 ± 19.3(8)	120.5 ± 16.7(6)	125.1 ± 20.6(6)
	Phenytoin	119.2 ± 19.1(4)	132.6 ± 15.7(8)	116.0 ± 12.1(6)	133.4 ± 23.5(6)
	% inhibition	—	—	—	—
	P =	N.S.	N.S.	N.S.	N.S.

[a] Phenytoin was administered as described in footnote *a*, Table I. Protein synthesis was measured in the mouse fetal bodies excluding palates as described under Materials and Methods.
[b] Values represent mean dpm ± SE; parentheses indicate numbers of individual litters examined.

TABLE VI. EFFECT OF PHENYTOIN ON RNA SYNTHESIS IN MATERNAL LIVER OF MICE[a]

Strain	Time (hr): Treatment	[^{3}H]Uridine incorporated/mg tissue (wet wt)[b] 3	24	48	72
A/J	Control	803.3 ± 62.7(6)	651.4 ± 97.5(8)	378.5 ± 84.9(15)	277.6 ± 80.2(6)
	Phenytoin	1047.3 ± 63.9(4)	841.1 ± 184.9(11)	604.9 ± 131.9(15)	356.9 ± 129.4(18)
	% increase	39.4	29.1	59.8	—
	P =	0.0003	0.045	7.5×10^{-6}	N.S.
B/6	Control	304.3 ± 69.6(8)	469.3 ± 124.0(18)	427.6 ± 133.8(9)	713.5 ± 81.8(6)
	Phenytoin	354.8 ± 32.5(14)	892.2 ± 138.1(18)	652.8 ± 257.9(1)	552.1 ± 187(10)
	% increase	16.6	90.1	52.7	—
	P =	0.038	0.032	0.042	N.S.

[a] Phenytoin was administered as described in footnote *a*, Table I. RNA synthesis was measured in the mouse fetal bodies excluding palates as described under Materials and Methods.
[b] Values represent mean dpm ± SE; parentheses indicate numbers of individual litters examined.

upon drug administration. However, it was only inhibited at 3 hr post-treatment in B6 whole embryo (Table IV). Protein synthesis was only inhibited at 48 hr post administration of phenytoin in A/J mouse fetal bodies. However, in B6 mouse embryo, it was unaffected (Table V).

RNA and protein synthesis in maternal liver. Maternal livers have an increase in RNA synthesis in both strains of mice at 3, 24, and 48 hr after drug administration. The maximal increase in maternal liver RNA synthesis appeared in the A/J strain at 48 hr (60%) and in the B6 strain at 24 hr (90%) (Table VI). Similarly, phenytoin stimulated maternal liver protein synthesis in the A/J strain at 3, 24, and 48 hr, while in the B6 strain the increase only occurred at 3 and 24 hr after drug administration (Table VII).

Discussion. The present results indicate that phenytoin produces a marked and persistent inhibition of RNA and protein synthesis in the fetal palates as well as in fetal bodies of A/J mice, a strain susceptible to phenytoin-induced cleft palate. However, in the B6 strain of mice, which is resistant to phenytoin-induced cleft palate, this drug produces only a slight and transient inhibition of fetal palatal and fetal body RNA synthesis, but does not affect protein synthesis. Thus, not only does this action of phenytoin resemble that produced by glucocorticoids in sensitive strains of mice (18), but also it is correlated with susceptibility of phenytoin-induced palatal clefting. Another parallel to glucocorticoid action is the discordant action of phenytoin on RNA and protein synthesis in the fetal palate and

TABLE VII. EFFECT OF PHENYTOIN ON PROTEIN SYNTHESIS ON MATERNAL LIVER OF MICE[a]

Strain	Time (hr): Treatment	[^{14}C]Leucine incorporated/mg tissue (wet wt)[b] 3	24	48	72
A/J	Control	391.4 ± 69.3(6)	384.9 ± 101.7(6)	566.0 ± 94.2(12)	624.8 ± 117.0(6)
	Phenytoin	689.7 ± 152.5(8)	734.7 ± 247.6(5)	966.8 ± 259.9(15)	521.7 ± 87.3(12)
	% increase	76.2	90.9	70.8	—
	P =	0.001	0.016	4×10^{-5}	N.S.
B/6	Control	447.8 ± 73.5(6)	416.4 ± 110.8(18)	431.5 ± 110.2(8)	400.1 ± 47.5(6)
	Phenytoin	491.8 ± 23.7(19)	538.4 ± 219.1(21)	679.7 ± 258.7(9)	417.6 ± 85.2(4)
	% increase	9.8	29.3	—	—
	P =	0.05	0.05	N.S.	N.S.

[a] Phenytoin was administered as described in footnote *a*, Table I. Protein synthesis was measured in the mouse fetal bodies excluding palates as described under Materials and Methods.
[b] Values represent mean dpm ± SE; parentheses indicate numbers of individual litters examined.

maternal liver. Triamcinolone also increases maternal hepatic RNA synthesis and decreases fetal palatal RNA synthesis in A/J mice (19). Although the precise mechanism by which inhibition of fetal palatal RNA and protein synthesis in A/J mice by phenytoin may lead to palatal clefting is unknown, the present results indicate that inhibition of palatal nuclear translation to specific proteins by phenytoin is associated with susceptibility.

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