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Phenytoin Teratogenicity in the Primary and Secondary Mouse Embryonic Palate Is Influenced by the *H-2* Histocompatibility Locus (41613)

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Abstract. Inbred and congenic strains of mice have been studied for susceptibility to phenytoin-induced cleft lip with or without cleft palate (CLP) and isolated cleft palate (CP). The role of genes linked to the *H-2* complex on chromosome 17 has been confirmed. Congenic strains with the A background have identical levels of spontaneous CLP, whereas those strains having the A background with the $H\text{-}2^a$ haplotype have significantly higher rates of induced CLP than their congenic partners with the $H\text{-}2^b$ or $H\text{-}2^s$ haplotype. No such significant difference in the degree of CLP produced by phenytoin is demonstrable in strains with the B background. Rates of isolated CP produced by phenytoin are significantly higher in strains with $H\text{-}2^a$ than in their congenic partner strains with either $H\text{-}2^b$ *or* $H\text{-}2^s$, whether the background is A or B.

Phenytoin is teratogenic in human embryos, commonly being associated with clefting of the primary palate, i.e., cleft lip with or without cleft palate (1–4). Clefts of the secondary palate (isolated cleft palate) can be induced by phenytoin in embryos of mice when injected on Days 11–13 of gestation and of rats on Days 12–15 (5–9). Given at an earlier time in gestation (Days 9 and 10), phenytoin also produces clefts of the lip (or lip and palate) in the susceptible Swiss Webster (7) and A/J (10, 11) mice, but not in the resistant C57BL/6J mice (10). Phenytoin crosses the placenta readily (12–14), and most of it can be recovered unmetabolized in the embryo (15). Very recently, we have shown that susceptibility to phenytoin-induced isolated cleft palate is influenced in part by genes in the *H-2* region of chromosome 17 as demonstrated by a significantly higher rate of induction of cleft palate by phenytoin in the B10.A strain as compared to that in its congenic partner strain, C57BL/10 (B10) (16). These two strains differ primarily at the *H-2* locus, but otherwise have a common genetic background of the B (C57BL) strain. Persons with susceptibility to clefts of the primary palate have long been thought to differ genetically from those susceptible to clefts of the secondary palate (17–19). In this paper we have studied the production of cleft lip (and palate) and isolated cleft palate by phenytoin in *H-2* congenic strains with either the A or B backgrounds. We show that production by phenytoin of cleft primary palate, cleft lip (and palate), is partially regulated by genes in the *H-2* locus in the presence of the A background only: whereas susceptibility to isolated cleft palate produced by phenytoin is regulated by genes in the *H-2* locus in the presence of either the A or B background.

Materials and Methods. Inbred and congenic mouse strains (A/J, A/Wy, A.BY, A.SW, B10.A, and B10) were obtained from the Jackson Laboratory, Bar Harbor, Maine, and were maintained on Purina Mouse Chow. Female mice were weighed and mated overnight with the appropriate males. Those females having plugs the next morning were again weighed on the day intended for injection. Those that had gained 1.5–2 g were considered pregnant and injected with either saline or phenytoin injectable, Elkins-Sinn, Inc., Cherry Hill, New Jersey (each milliliter contains phenytoin sodium 50 mg, propylene glycol 0.4 ml, and alcohol 0.1 ml in water for injection (pH adjusted to 12 with sodium hydroxide)).

For cleft lip (palate) all strains were injected intramuscularly with phenytoin 100 mg/kg (or saline) either at 9 AM on 10 days, 9 and 10 days, or 8, 9, and 10 days after the plug.

For isolated cleft palate both the A and B strains were injected intramuscularly with phenytoin 50 mg/kg (or saline) at 9 AM in the mornings of 11, 12, 13, and 14 days after the plug.

The fetuses were removed on Day 18, and

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the primary and secondary palates examined under a dissecting microscope.

It has been suggested that the litter rather than the fetus is the proper unit of measurement in teratology (20), accordingly all tests of significance were performed by the Mann–Whitney *U* test. To analyze the effect of phenytoin on cleft lip with or without cleft palate, the percentage of clefts of the primary palate in each litter was corrected by subtracting the rate of spontaneous clefting of the primary palate characteristic of the particular strain from the total rate of clefting of the primary palate in that litter. To analyze for the effect of phenytoin on the rate of clefting of the secondary palate those fetuses with clefts of the primary palate which must have occurred prior to the time of injection (21) were omitted from consideration of the rates of induced clefting.

Results. In order to determine the rate of spontaneous appearance of cleft palate, the fetuses from mothers that had received only vehicle were examined (Table I). None of the strains had the occurrence of isolated cleft palate. Each of the four strains with the A genetic background had some incidence of spontaneous cleft lip with or without cleft palate (CL(P)), while each B strain had none. Further, the rate of spontaneous cleft lip (palate) does not vary significantly among the congenic strains, A.BY (*H-2*^{b}) and A.SW (*H-2*^{s}), and their inbred donor strain A/Wy (*H-2*^{a}) (22). However, each of the congenic strains with the A background has a significantly higher rate of spontaneous CL(P) than the A/J strain ($P < 0.007$ in each case). These results indicate the importance of non-*H-2*-linked genes in spontaneous CL(P). Despite the A/J's *H-2*^{a} haplotype, identical to that of the A/Wy and the similar A ancestor strain origin of A/J and A/Wy (23), some genes presently differ in the A background which confer a higher rate of spontaneous CL(P) in the congenic strains. This can only be explained by genes occurring by spontaneous mutation in the A/Wy and A/J strains.

TABLE I. FREQUENCIES OF SPONTANEOUS CLEFT LIP AND PALATE IN INBRED AND COGENIC STRAINS OF MICE

Strain	*H-2*	No. Litters	Resorptions		Fetuses		
			No.	%	Total No.	Cleft lip* No.	%
A/J	a	19	16	10	139	13	9
A/Wy	a	16	14	11	117	25	21
A.BY	b	17	15	14	92	25	27
A.SW	s	21	15	10	134	35	26
B10	b	5	4	10	45	0	0
B10.A	a	8	3	4	76	0	0

* With or without cleft palate.

Table II presents the data on phenytoin-induced CL(P) in the various strains. A/Wy (*H-2*^{a}) mice have a significantly higher rate of induced CL(P) than either of its congenic partners, A.BY (*H-2*^{b}) ($P < 0.005$) or A.SW (*H-2*^{s}) ($P < 0.04$). The A/J (*H-2*^{a}) strain also shows high susceptibility to phenytoin-induced CL(P) when compared to the same two strains ($P < 0.05$ in each case). Thus, evidence for a gene(s) in the *H-2* complex conferring susceptibility to phenytoin-induced CL(P) is provided; *H-2*^{a} conferring more susceptibility than *H-2*^{b} or *H-2*^{s}. No significant difference is detected between the phenytoin-induced rates of CL(P) in the A/Wy and A/J strains indicating that gene differences in these strains only affect spontaneous clefting frequency. The *H-2*^{b} and the *H-2*^{s} alleles seem to provide no difference in resistance to phenytoin-induced CL(P).

The data on the congenic B strain partners indicate that phenytoin also produces CL(P) in these strains, but the critical period appears to be earlier than that shown for the A/J strain (10, 11, 21) (Table II). The degree of CL(P) in the B10.A strain is not significantly different from that in the B10 strain indicating that high susceptibility to phenytoin-induced CL(P) is the result of interaction between gene(s) within the *H-2* complex and non-*H-2* genes present only in the A strains. The data do not permit a precise localization of individual genes within the *H-2* locus that control this susceptibility trait.

The frequency of phenytoin-induced isolated cleft palate is significantly higher in the strains having the *H-2* haplotype irrespective of whether the background is A ($P < 0.04$) or B ($P < 0.04$) (Table III). As in the case of CL(P) neither the *H-2*^{b} nor *H-2*^{s} allele seems to provide more resistance to phenytoin-in-

TABLE II. FREQUENCIES OF PHENYTOIN-INDUCED CLEFT LIP (PALATE) IN INBRED AND *H-2* CONGENIC STRAINS OF MICE

Strain	*H-2*	Background	Days	No. Litters	Resorptions		Fetuses			
							Total	With cleft lip		
					No.	%	No.	No.	%	Corrected %*
A/J	a	A	10	14	26	19	108	75	69	60
A/Wy	a	A	10	10	14	17	68	54	79	58
A/BY	b	A	10	22	22	17	110	76	69	42
A.SW	s	A	10	13	16	16	84	52	62	36
B10.A	a	B	10	5	7	14	43	1	2	2
B10.A	a	B	9, 10	9	10	11	83	3	4	4
B10.A	a	B	8, 9, 10	9	18	21	67	6	9	9
B10	b	B	8, 9, 10	4	9	24	28	4	14	14

* Corrected percentage = induced percentage–spontaneous percentage (from Table I).

duced cleft palate than the other. Thus, gene(s) in the *H-2* complex on chromosome 17 confer sensitivity to phenytoin-induced cleft palate with both A and B backgrounds.

Discussion. The data concerning the production of isolated cleft palate in the present report has extended our previous results (16) by showing that genes in the *H-2* complex affect susceptibility to phenytoin-induced cleft palate not only in mice of the B background, but in mice of the A background as well. Our earlier mapping studies (24) have shown that the phenotype of susceptibility to either cortisone- or phenytoin-induced isolated cleft palate requires two genes at opposite ends of the *H-2* complex to complement one another. Non-*H-2* genes in the A background are also involved, since the rate of phenytoin-induced clefting is significantly higher in the A.BY ($H\text{-}2^b$) strain than in the B10 ($H\text{-}2^b$) strain ($P < 0.03$).

It is of interest that with respect to CL(P) the potent glucocorticoid, triamcinolone hexacetonide, produces CL(P) only in the A/J strain on Day 8 but not in either the B10.A or B10 strain at this time (25). This finding is consistent with the idea that susceptibility to triamcinolone hexacetonide-induced CL(P) may also be influenced by an interaction of the *H-2* locus with a non-*H-2* gene in the A background, but a comparison of the effects of this glucocorticoid in the A/Wy and A.BY strains must be made before this can be proved.

The qualitative similarity of the effects on palatal development produced by phenytoin in man and experimental animals to those produced by glucocorticoids is noteworthy. The present report provides evidence that susceptibility to both teratogens of the primary and secondary palate may be affected by some of the same genes. Both cortisone and triam-

TABLE III. FREQUENCIES OF PHENYTOIN-INDUCED ISOLATED CLEFT PALATE IN *H-2* CONGENIC STRAINS OF MICE

Strain	*H-2*	Background	Days	No. Litters	Resorptions		Fetuses				
							Total	Cleft lip (palate)		Cleft palate	
					No.	%	No.	No.	%	No.	%
A/J	a	A	11–14	20	40	21	148	9	6	80	57
A.BY	b	A	11–14	15	9	18	51	9	18	14	31
A.SW	s	A	11–14	11	6	7	75	20	27	16	29
B10.A	a	B	11–14	14	25	18	117	0	0	26	22
B10	b	B	11–14	9	1	2	63	0	0	1	2

cinolone reduce the frequency of fetal movements during palatal development (26) and phenytoin also produces a diminution of fetal muscular movements during palatal differentiation (27). Both cortisone and phenytoin delay shelf elevation from a vertical to a horizontal position (27). When phenytoin is given in conjunction with cortisone, it does not alter the frequency of isolated cleft palate induced by the latter suggesting a common pathway (9). Recently, in a study of the comparative teratogenicity of cortisone and phenytoin in mice a probit analysis indicated that both agents may have an identical mechanism (28).

The biochemical mechanism that would account for these genetic data remains to be elucidated. It has been reported that B10.A mice have a higher level of palatal cortisol binding cytosolic receptors than do B10, suggesting that an *H-2*-linked gene affects the structure or quantity of this receptor (29). More recently, this difference has been confirmed by the use of dexamethasone, which has a greater specificity of binding than cortisol (30). It was observed in recombinant inbred strains that the genes controlling receptor level mapped in the same loci within the *H-2* complex as did those controlling susceptibility to cortisone-induced cleft palate (30). It has also been shown that this strain difference could not be demonstrated in liver (30, 31), which is in agreement with observations of tissue-specific variation in cortisol receptors in rats (32). In cultured human fetal palatal cells phenytoin interferes with the binding and translocation of [^{3}H]dexamethasone to the nucleus (33). In earlier studies blocking of and translocation of [^{3}H]dexamethasone directly to A/J palatal cell cytosol receptors could not be demonstrated at a high dose of phenytoin (34). We have found, however, that by using Na_2CO_3 to solubilize the phenytoin (35), specific [^{3}H]-phenytoin binding to the glucocorticoid receptor in lungs and liver of A/J mice is competitively inhibited by dexamethasone. However, the biochemical events that control palatal closure could be very complex, and it is by no means clear whether an *H-2*-linked effect on glucocorticoid receptors will account for the genetic differences in susceptibility to phenytoin.

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