Teratogenic Potential of Phenytoin in Different Strains of Mice

D. K. Hansen and M. E. Hodes
Department of Medical Genetics
Indiana University School of Medicine
Indianapolis, Indiana 46223

Introduction

Phenytoin (PHT) is an anticonvulsant drug which is commonly used in the treatment of epilepsy. Evidence was first introduced in 1967 which suggested that PHT is teratogenic in humans (15). Subsequent studies demonstrated that women with epilepsy who had taken anticonvulsant drugs during their pregnancies had more frequent malformations in their offspring than either women with epilepsy who had not taken drugs or non-epileptic women (9). Hanson and Smith (6) described the fetal hydantoin syndrome, a pattern of malformation in children whose mothers had taken PHT during their pregnancies. It has been estimated that 11% of the children exposed to PHT *in utero* have the fetal hydantoin syndrome and an additional 31% have some of the features of the syndrome (7). A genetic predisposition for such drug-induced malformations had been suggested by several authors (3,7,9). However, evidence on this point is unclear.

Several studies (2,4,8,14,22) have shown that PHT causes an increase in the incidence of cleft palate in various strains of mice. Gibson and Becker (5) speculated that differences in the genetic constitutions of the two strains that they examined could account for the differences in susceptibility to teratogenesis that they observed. The current study was undertaken to examine the possible role of such genetic factors.

Materials and Methods

A mouse colony was established from each of the following strains: A/J, AKR/J, BALB/c, C57BL/6, DBA/2J, and ICR. The animals were housed in plastic cages on hardwood bedding and were allowed food and tap water *ad libitum.* Humidity, temperature, and light: dark cycle were held constant throughout all experiments. Virgin females 7 to 12 weeks of age were housed with males (one male to two or three females). Copulation was ascertained by the presence of a vaginal plug which determined day 0 of gestation. Pregnant females were removed from the cage and were housed together. PHT dissolved in 0.05% sodium hydroxide at a concentration of 10 mg/ml was injected intraperitoneally on gestation days 10, 11, and 12. Control animals received the vehicle by intraperitoneal injection. In the initial experiment the dosage of PHT was different, and the drug was administered at 50, 75, or 100 mg/kg body weight. On day 18 of pregnancy, the pregnant females were sacrified by cervical dislocation, the uterine horns were exteriorized, and the fetuses were removed. Each fetus was weighed and examined for gross abnormalities. One-third of all fetuses were fixed in 95% alcohol prior to clearing in 1% potassium hydroxide and staining in alizarin red S (Eastman Organic Chemicals) (24). These animals were later measured for crown-rump-length and transumbilical distance. The remaining fetuses were fixed in Bouin's fluid, sectioned and examined for soft tissue abnormalities.

Differences between drug treated and control animals within a strain in number of resorptions, stillbirths, and abnormal fetuses (total includes all animals

with cleft lip with/without cleft palate) were tested for significance by X^2 test. Differences for continuous variables such as body weight, length, and transumbilical distance were examined by Student's t test (21). The five percent level of significance was used in all studies.

Results

In order to determine if there is a dosage effect of PHT on malformation frequency, the drug was administered to pregnant ICR mice at 50, 75, or 100 mg/kg body weight. The highest dose was toxic to the mothers and was not further analyzed. PHT did not significantly affect the frequency of resorptions or stillbirths at either 50 (X^2 = 1.87) or 75 (X^2 = 1.98) mg/kg. The drug did not cause a significant difference in the frequency of total malformations (X^2 = 0.26) or orofacial anomalies (X^2 = 0) at 50 mg/kg. However, at the higher dose, there was a significant increase in total malformations (X^2 = 14.76, $P < .01$). This increase can be accounted for by the significant increase in orofacial anomalies (X^2 = 33.24, $P < 0.01$). Therefore, 75 mg/kg was the dose chosen for all subsequent experiments.

Tables 1 and 2 show the results of the strain susceptibility experiments. The frequency of resorptions was most affected by the drug; four of the six strains examined demonstrated significant increases in this variable following drug treatment. Frequency of stillbirths and transumbilical distance were the variables least affected by drug treatment.

TABLE 1. *Percentage of fetal abnormalities in 6 strains of mice after administration of phenytoin (75 mg/kg) to the mothers. Resorptions and stillbirths are expressed as percentage of total number of implants; total malformations and orofacial anomalies are expressed as percentage of number of live fetuses. Numbers in parentheses are total number of implants in that treatment group. Twenty pregnant females were utilized in each treatment group.*

	Resorptions	Stillbirths	Total Malformations	Orofacial Anomalies
A/J				
Control (177)	13.30	2.45	15.34	6.13
Treated (162)	23.81*	2.08	30.21*	21.88**
AKR/J				
Control (197)	13.20	2.29	4.57	1.04
Treated (218)	21.56*	2.29	1.71	0.47
BALB/c				
Control (208)	18.37	2.50	1.67	0.00
Treated (194)	21.09	1.72	3.45	0.00
C57BL/6				
Control (160)	7.50	1.33	5.33	0.63
Treated (162)	8.02	3.87	3.87	0.00
DBA/2J				
Control (160)	7.50	6.33	6.33	0.67
Treated (148)	25.00**	3.48	11.30	1.39
ICR				
Control (216)	4.62	1.44	4.31	0.00
Treated (231)	9.52*	0.48	6.67	3.04*

*Signficant at 0.05 level
**Significant at 0.01 level

TABLE 2. *Changes in selected measurements in the fetuses of 6 strains of mice after administration of phenytoin (75mg/kg) to the mothers.*

STRAIN		WEIGHT(g) (Mean ± SD)	CROWN-RUMP(mm) (Mean ± SD)	TRANSUMBILICAL(mm) (Mean ± SD)
AJ				
	Control	0.99 ± 0.16	20.9 ± 9.1	5.5 ± 2.4
	Treated	0.83 ± 0.07**	15.5 ± 11.4	4.2 ± 3.1
AKR/J				
	Control	1.10 ± 0.21	22.3 ± 8.2	5.7 ± 2.0
	Treated	0.99 ± 0.13*	21.9 ± 8.0	5.6 ± 2.0
BALB/C				
	Control	1.15 ± 0.16	24.7 ± 1.7	6.5 ± 0.7
	Treated	1.01 ± 0.22*	22.7 ± 2.0*	6.3 ± 0.6
C57BL/6				
	Control	1.04 ± 0.19	21.4 ± 5.6	5.4 ± 1.9
	Treated	0.98 ± 0.19	22.1 ± 5.8	5.7 ± 1.5
DBA/2J				
	Control	0.90 ± 0.08	16.8 ± 10.2	4.3 ± 2.6
	Treated	1.00 ± 0.20	20.9 ± 7.4	5.4 ± 1.9
ICR				
	Control	1.19 ± 0.22	26.3 ± 2.0	6.7 ± 0.3
	Treated	1.16 ± 0.14	27.0 ± 2.1	6.7 ± 0.4

*Significant at 0.05 level
**Significant at 0.01 level

A/J mice were the strain most susceptible to the effects of the drug. This strain demonstrated signficiant increases in the frequencies of resorptions, total malformations, and orofacial anomalies and a significant decrease in fetal weight following drug treatment. C57BL/6 mice were the least susceptible and did not demonstrate signficiant differences between control and treated animals for any of the variables tested.

Discussion

The studies of PHT-induced teratogenesis in mice that have been done in the past have varied in their choice of strain as well as in other aspects of methodology. Particularly bothersome are the different methods of administration of the drug to the pregnant mice. The plasma concentration of the drug is related to its method of administration in humans (16), and there is no reason to suspect that this is not also true for mice. Since this variable has been different in most of the studies, it is extremely difficult to compare their conclusions. In order to reach valid conclusions on the effects of strain and thus of inheritance, it is necessary to examine teratogenicity in several strains under constant conditions. With this in mind, we tested the teratogenic potential of PHT in six different strains of mice under very carefully controlled conditions. We found that of the 6 strains we examined, the A/J was the most susceptible to the effects of PHT and the C57BL/6 was the most resistant. Johnston *et al.* (10) examined only these two strains and found the same order of susceptibility. We currently are testing the teratogenicity of the drug in F_1 hybrids resulting from reciprocal crosses involving A/J and C57BL/6 mice.

The mechanism of PHT teratogenesis is unknown. The drug can be metabolized by the liver microsomal cytochrome P-450 monooxygenase system to a reactive epoxide (1) which is capable of binding to tissue macromolecules (13). Activation of other polycyclic aromatic hydrocarbons by metabolism via this pathway can lead to an increase in teratogenesis (19, 20), mutagenesis (12), carcinogenesis (11), and toxicity (17, 18, 23). At present, the role of genetic factors in these aspects of metabolism and teratogenesis is unclear.

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