

SHORT COMMUNICATION

DIPHENYLHYDANTOIN EFFECTS IN BALB C MOUSE BONE MARROW CELLS: CYTOGENETIC ASPECTS

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ABSTRACT

The effect of the anticonvulsant diphenylhydantoin on chromosomes of Balb C mouse bone marrow cells was evaluated. An increase in the frequency of aneuploid cells was observed when a teratogenic schedule of treatment was used. The therapeutic levels did not induce the same cytogenetic abnormality. No clastogenic effect resulted from either treatment.

INTRODUCTION

Diphenylhydantoin (DPH) has been extensively used as anticonvulsant in the treatment of epileptic patients. However, this drug has several side effects including a high incidence of certain types of tumors, coagulation defects, gingival hyperplasia, and it probably affects the normal development and expression of humoral immune function (Iivanainen and Savolainen, 1983). DPH is known to be a teratogenic agent (Albengres and Tillement, 1983), although its mechanism of teratogenicity is still unknown. The drug has been associated with congenital abnormalities, and the "Fetal Hydantoin Syndrome", including over 50 types of malformations, has been reported in humans (Hanson and Smith, 1975). The pattern of malformations induced by DPH treatment in mice is similar to that observed in the human syndrome, i.e. delayed ossification, cardiac and renal anomalies, ocular defects and cleft palate (Finnel and

Chernoff, 1984). The teratogenicity of DPH was also described in rats (Gibson and Becker, 1968), cats (Khera, 1979), rabbits (McClain and Langhoff, 1980) and mice (Harbison and Becker, 1969).

The increased risk of tumors associated with DPH has been attributed to exposure *in utero*, since neuroblastomas (Pendergrass and Hanson, 1976; Sherman and Roizen, 1976; Allen *et al.*, 1980; Ehrenbard and Chaganti, 1981), ganglioneuroblastoma (Seeler *et al.*, 1979), melanotic ectodermal tumor (Jimenez *et al.*, 1981) and ependymoblastoma (Lipson and Bale, 1985) have been reported in children with "Fetal Hydantoin Syndrome".

Considering the controversial results obtained when DPH was evaluated at the cytogenetic level (Gebhart, 1984), additional data are important to elucidate this effect.

In the present study, mouse bone marrow cells were analyzed cytogenetically after *in vivo* DPH treatment. The embryotoxicity of DPH was also evaluated.

MATERIAL AND METHODS

Animals and DPH treatment

Adult Balb C females were submitted to 2 schedules of treatment with DPH (purchased from the Pharmacy of HC-FMUSP):

1. Therapeutic schedule: 0.48 mg DPH, 3 days a week given orally for 2 months. This drug concentration is similar to that used for epileptic patients (Toman, 1970). In this group the females were mated 15 days before sacrifice.

2. Teratogenic schedule: pregnant females were treated intraperitoneally with 3 doses of 50 or 100 mg DPH/kg body weight on days 11, 12 and 13 of pregnancy (Harbison and Becker, 1969) and sacrificed on day 14.

Parallel control groups were treated with the vehicle, 0.05% NaOH.

Observation of the fetuses

After laparotomy the fetuses were exposed and examined for external anatomical malformations, the number of dead and abnormal fetuses per group was scored and compared statistically by the χ^2 test.

Metaphase preparations

Bone marrow cell preparations were essentially performed by the method described by Ford and Hamerton (1956). One hundred metaphases per animal, prepared by the air-drying technique and conventionally stained with Giemsa, were analyzed for numerical and structural abnormalities and the data were analyzed

statistically by standard variance analysis. When a difference at the 1% level of significance was detected, the data were further analyzed by a multiple comparison test. (Student-Newman-Keuls test).

RESULTS AND DISCUSSION

Chronic DPH treatment of Balb C females for two months at therapeutic concentrations (Toman, 1970) is effective in inducing fetal malformations. Figure 1 shows that 41% of DPH-exposed embryos were abnormal. However, the drug did not increase significantly the frequency of chromosomal aberrations in female bone marrow cells after this treatment schedule (Figure 2). The chromosomal aberration levels did not change even after 5 months of DPH treatment (data not shown).

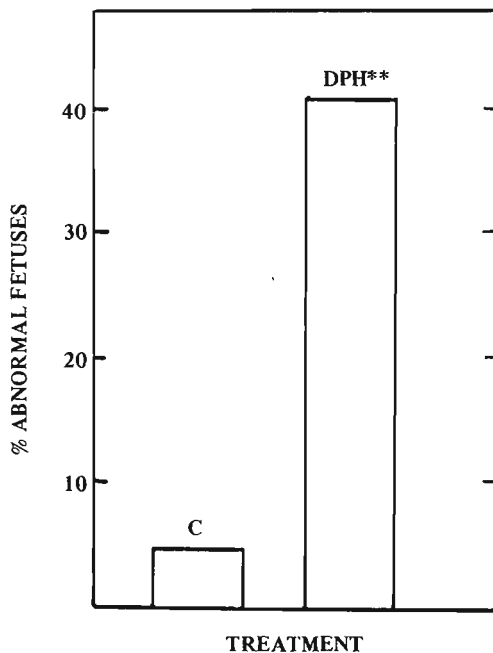


Figure 1 - Incidence of fetal malformations in Balb C females treated orally with 0.48 mg DPH 3 days a week for 2 months. The data were obtained from 5 females per treatment group. ** Significantly different from control (C) ($P < 0.01$).

When the animals were submitted to a teratogenic treatment schedule (Harbison and Becker, 1969) similar results were observed: after 3 doses of 50 or 100 mg DPH/kg body weight the abnormal fetuses frequency increased from 0% (control) to 23.4% and 31.2%, respectively (Figure 3).

No significant increase in the frequency of structural chromosomal abnormalities was observed in bone marrow cells of animals under these treatment schedules (Table I). Nevertheless, clastogenic effects have been reported to occur in human

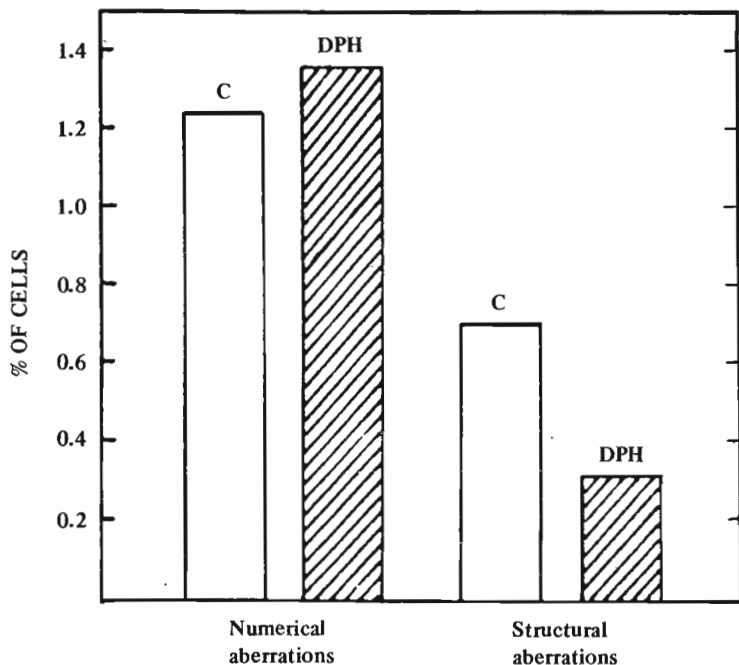


Figure 2 - Chromosomal findings in bone marrow cells of pregnant Balb C females chronically treated with DPH as described in Figure 1. The data were obtained from 5 animals per treatment group. C, control.

Figure 3 - Incidence of fetal malformations in Balb C females treated intraperitoneally with 3 doses of 50 or 100 mg DPH/kg body weight on days 11, 12 and 13 of pregnancy. The data were obtained from 10 females per group. Significantly different from control (C) (* $P < 0.002$ and ** $P < 0.001$).

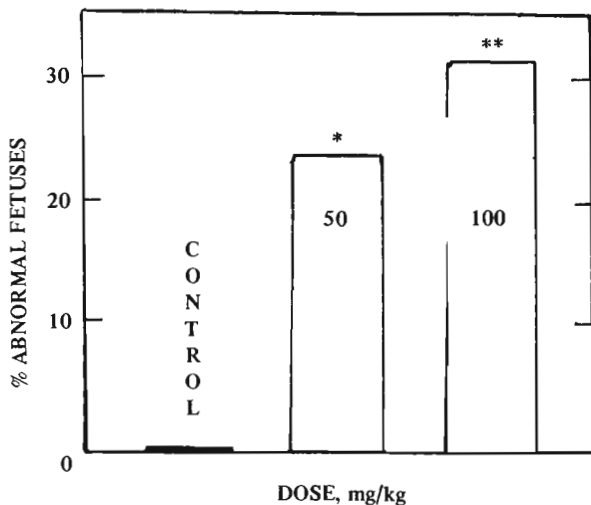


Table I - Chromosomal findings in bone marrow cells of Balb C mice treated with diphenylhydantoin.^a

Treatment	Animal	Total no. of metaphases analyzed	Frequency of abnormal metaphases (%)	
			Numerical	Structural
Control	1	100	1.0	1.0
	2	100	2.0	1.0
	3	40	0.0	0.0
	4	100	4.0	0.0
	5	100	2.0	1.0
	6	100	0.0	0.0
	7	100	2.0	1.0
	8	100	0.0	0.0
	9	100	1.0	1.0
	10	100	0.0	0.0
	Total	940	1.3	0.5
50 mg DPH/kg body weight	1	100	7.0	3.0
	2	100	3.0	2.0
	3	100	4.0	1.0
	4	100	6.0	0.0
	5	100	4.0	0.0
	6	100	5.0	2.0
	7	100	6.0	2.0
	8	100	4.0	1.0
	9	100	7.0	0.0
		Total	900	5.1*
100 mg DPH/kg body weight	1	100	3.0	0.0
	2	59	1.7	1.7
	3	91	3.3	2.2
	4	100	5.0	0.0
	5	45	4.4	0.0
	6	49	0.0	2.0
	7	100	6.0	0.0
	8	100	6.0	2.0
	9	100	5.0	0.0
	10	100	6.0	0.0
	Total	844	4.4*	0.7

^a Treatment schedule described in Figure 3.* Significantly different from control ($P < 0.005$).

lymphocytes as a consequence of DPH therapy (Ayraud *et al.*, 1968; Grosse *et al.*, 1972; Ayraud *et al.*, 1974; Herha and Obe, 1976).

The treatment with teratogenic doses of DPH increased significantly ($P < 0.005$) the frequency of aneuploid metaphases (Table I). We also obtained similar effect in human and mouse fibroblast cells treated *in vitro* with high doses (100-200 $\mu\text{g/ml}$) of DPH (Oliveira and Machado-Santelli, in press). These results agree with those of de Toni *et al.* (1966), Ayraud *et al.* (1968) and Márquez-Monter *et al.* (1970) which indicate an increase of polyploid and heteroploid cells in epileptic patients and their children after DPH treatment. Although in mice it is necessary to use doses higher than the therapeutic ones to induce aneuploidy, we assume that the major effect is the same in humans and in mice.

This aneugenic effect may be the consequence of a mitotic arresting effect of DPH since it affects the polymerization of mitotic spindle fibers *in vitro* (MacKinney *et al.*, 1978a,b; Oliveira and Machado-Santelli, in press).

Therefore DPH can be considered as a spindle poison and this aspect must be taken into account in the carcinogenic, mutagenic and teratogenic evaluation of anti-epileptic drugs.

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RESUMO

O efeito do anticonvulsivo difinilhidantoína (DPH) foi analisado em cromossomos de células de medula óssea de camundongos Balb C. Foi observado um aumento na frequência de células aneuploides em animais submetidos a doses teratogênicas da droga. Esse efeito não foi encontrado após o tratamento crônico com dose terapêutica de DPH. O DPH não teve efeito clastogênico nos esquemas de tratamento utilizados.

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