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In vivo dominant lethal effect of pyrimethamine in male mouse germ cells

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Pyrimethamine is used for treatment of malaria and toxoplasmosis. The embryotoxicity and clastogenicity of pyrimethamine is known and our aim was to investigate its dominant lethal effect *in vivo*. For this purpose, we used three groups of Swiss-albino male mice and a control group. We injected males with doses of 16, 32 or 64 mg/kg pyrimethamine and housed them with 10 females/male for each mating interval. Females were sacrificed and their uteri were evaluated for dominant lethality. As a result of this study we found that pyrimethamine induced dominant lethal mutations in the third, fourth and sixth weeks at the 64 mg/kg dose level, without the effect being dose-dependent. We conclude that pyrimethamine is a suspected germ cell mutagen.

Introduction

Pyrimethamine (PYM) is an inhibitor of dihydrofolate reductase (Goodman-Gilman *et al.*, 1990) which is used in antimalarial regimens for both treatment and prophylaxis (Chung *et al.*, 1993). In human usage, the therapeutic dose of PYM is 50 mg/day and an ~15 day treatment period is recommended (Meyers *et al*., 1974). However, for prophylaxis of malaria, it may be used over a longer period. Pyrimethamine has been freely sold since 1952 for prophylaxis of malaria and the numbers of prophylactic doses taken until 1983 run into millions (Peters, 1983). Also, pyrimethamine was at one time included in several chemoprophylactic campaigns in Africa and South-East Asia under the World Health Organisation (Peters, 1983).

Embryotoxicity of PYM, especially teratogenic effects, has been reported for rats (Tsunematsu *et al.*, 1990), hamsters (Sullivan and Takacs, 1971), pigs (Hayama and Kokue, 1985) and humans (Harpey *et al.*, 1983).

Genotoxic effects of PYM have been determined in cultured human lymphocytes (Egeli and Erdoğan, 1991; Egeli and Tunca, 1997), in cultured Chinese hamster lung (CHL) cells (Ono *et al.*, 1994; Ono and Yoshimura, 1996) and in cultured Cl-1 Chinese hamster cells (Antoccia *et al*., 1991).

PYM induces aneuploidy in rat bone marrow cells (Cimino *et al.*, 1986) and in rat oocytes (Chebotar, 1980). Awoniyi *et al.* (1993) suggested that a 400 mg/kg PYM treatment causes reduced fertility in male rats. Additionally, it was reported that PYM produces reversible infertility in Swiss-Webster male mice (Cosentino *et al.*, 1990).

In the light of this fertility and cytogenetic data, our aim was to determine if PYM exerts an *in vivo* dominant lethal effect on male mouse germ cells.

Materials and methods

Animals

Swiss-albino mice were obtained from the Test Animals Breeding Center of the University of Uludağ (Bursa, Turkey). They were 8 months old and housed in cages constructed of stainless steel with a bedding of wood shavings. Animals were fed with fresh standart pellet (The Chow Company, Bursa, Turkey) and water *ad libitum*. They were housed at a temperature of 18 ± 0.3°C.

Chemicals

PYM was purchased from Sigma Chemical Co. (St Louis, MO; no. 88F0320) and the purity of this compound was >99%. It was dissolved in 99% ethanol. The maximum amount of ethanol was 0.05 ml/mouse.

Mating procedure

We used three groups of male mice, each group consisting of five mice. These three groups were injected i.p. at doses of 16, 32 or 64 mg PYM/kg. It is reported that the maximum tolerated dose of pyrimethamine was 50 mg/kg for a single oral administration (Ono and Yoshimura, 1996). In our study we treated mice with up to 80 mg PYM/kg i.p. We observed toxic effects and deaths at this dose level. Five control male mice were injected with 0.05 ml ethanol only.

During weeks 1–7 inclusive, each male in each group was housed with two virgin females of the same strain. At the end of the week the females were replaced with fresh females. Eighteen days after first introducing the male, females were killed. The uteri were evaluated for dominant lethality by counting total implants, early deaths, late deaths and live implants. Early and late deaths were not listed separately; we combined these parameters as post-implantation losses (Ashby and Clapp, 1995). The frequency of induced dominant lethal mutations was calculated as 1 – (live implants per female of the test group/live implants per female of the control group)×100. Corpora lutea were not counted.

Statistical analysis

The level of significance between the post-implantation loss per female in the treated and control groups was determined with the Mann–Whitney *U* test (Zarr, 1984).

Results

The results of the dominant lethal test are summarized in Table I. All females that had implants were classified as fertile. The results indicate that PYM induced post-implantation loss in the third, fourth and sixth weeks with the highest dose (64 mg/kg) and these differed significantly from the control value (Table I). With the lower dose levels (16 and 32 mg/kg) no induction of dominant lethals could be observed ($P > 0.05$). A dose of 64 mg/kg significantly induced dominant lethal mutations during the mating intervals 15–21, 22–28 and 36–42. days ($P < 0.05$) (Figure 1).

Discussion

In the mouse, the period of spermatogenic maturation is generally 8 weeks (Shelby and Tindall, 1997). In the present study the dominant lethality resulting from exposure to PYM is significant on days 14–21, 22–29 and 35–42 after mating with the highest dose level (64 mg/kg). The mating intervals (days 14–21, 22–29 and 35–42) indicate that damage that can

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Table I. Results of dominant lethality in male mouse germ cells treated with pyrimethamine

Dose (mg/kg)	Mating interval (days)	No. mating females	No. females with implants (%)	Total implants/female[a]	Live implants/female[a]	Post-implantation loss/female[a]	Dominant lethal mutation (%)
Control	1–7	10	80	12.62 (0.73)	12.50 (0.68)	0.12 (0.12)	
	8–14	10	100	12.40 (0.66)	11.70 (0.63)	0.70 (0.26)	
	15–21	10	100	10.60 (0.40)	10.50 (0.45)	0.10 (0.10)	
	22–28	10	100	11.70 (0.42)	11.30 (0.30)	0.40 (0.26)	
	29–35	10	100	11.60 (0.42)	11.00 (0.51)	0.60 (0.30)	
	36–42	10	90	12.11 (0.42)	12.00 (0.37)	0.11 (0.11)	
	43–49	10	90	12.00 (0.64)	11.55 (0.66)	0.44 (0.17)	
16	1–7	10	90	9.44 (0.70)	8.44 (0.70)	1.00 (0.44)	24
	8–14	10	90	9.44 (0.70)	8.44 (0.70)	1.00 (0.44)	36
	15–21	10	90	10.00 (0.64)	9.22 (0.64)	0.77 (0.27)	21
	22–28	10	90	10.00 (0.64)	9.22 (0.64)	0.77 (0.27)	27
	29–35	10	100	11.00 (0.55)	10.10 (0.54)	0.90 (0.31)	9
	36–42	10	100	11.00 (0.55)	10.11 (0.55)	0.90 (0.32)	7
	43–49	10	80	9.37 (1.26)	9.12 (1.27)	0.25 (0.25)	30
32	1–7	10	70	9.57 (0.75)	9.28 (0.64)	0.25 (0.16)	35
	8–14	10	90	9.77 (0.86)	8.77 (0.96)	1.00 (0.33)	33
	15–21	10	70	10.57 (0.29)	10.42 (0.29)	0.14 (0.14)	31
	22–28	10	100	7.10 (1.08)	6.60 (1.02)	0.50 (0.26)	42
	29–35	10	100	8.30 (0.93)	7.80 (0.78)	0.50 (0.30)	30
	36–42	10	100	11.20 (0.77)	10.50 (0.88)	0.70 (0.30)	3
	43–49	10	100	8.70 (0.76)	8.30 (0.66)	0.40 (0.22)	21
64	1–7	10	80	12.00 (0.62)	10.37 (0.70)	1.50 (0.50)	17
	8–14	10	100	10.30 (0.63)	9.60 (0.63)	0.70 (0.30)	18
	15–21	10	70	9.00 (0.30)	7.14 (0.50)	1.85 (0.59)[b]	53
	22–28	10	70	7.85 (0.40)	6.14 (0.45)	1.71 (0.28)[b]	63
	29–35	10	70	9.85 (1.14)	8.71 (1.16)	1.14 (0.40)	45
	36–42	10	90	7.11 (1.19)	5.22 (1.71)	1.88 (0.97)[b]	57
	43–49	10	60	10.33 (0.55)	9.66 (0.71)	0.66 (0.21)	45

[a]Numbers in parentheses are standard errors of means.
[b]Significant at the $P < 0.05$ level.

result in dominant mutation is induced specifically from spermatids to spermatogonial cells (Shelby and Tindall, 1997). A marked reduction in total implants was observed with the same dose. The results indicate that induction of mutation is due to post- and pre-implantation loss.

PYM is a folate antagonist and delays replication by inhibiting folate reductase enzyme and by preventing formation of thymidilate, which is used in DNA synthesis (Meyers *et al.*, 1974; Goodman-Gilman *et al.*, 1990). As a result, lesions in DNA are produced, resulting in gaps and breaks in chromosomes (Egeli and Erdoğan 1991; Aydemir and Bilaloğlu, 1996).

In accordance with the experience of Brewen *et al.* (1975), it was found that evidence of broken chromosomes at the first meiotic division correlated with dominant lethality. It is expected that the broken chromosomes were eventually lost at anaphase, resulting in a monosomic embryo that subsequently died *in utero*.

In one of the our previous studies, it was reported that PYM induced acentric fragments and breaks at the 80 and 120 mg/kg dose levels in the spermatocyte stage of Swiss-albino male mice (Aydemir and Bilaloğlu, 1996).

In rats, PYM significantly reduced testis and epididymis weights, testicular and epididymal sperm counts and fertility (Awoniyi *et al.*, 1993). Awoniyi *et al.* (1993) found that PYM induced a decline in pachytene spermatocytes and round spermatids as well as atrophy of ~30% of the seminiferous tubules in rats in this study. Cosentino *et al.* (1990) observed similar data in their study. According to this study PYM decreased sperm production and seminiferous tubule diameter by acting on spermatogenesis in male mouse (Cosentino *et al.*, 1990). Additionally, they suggested that this compound acted particularly on early to mid spermatogenesis. We also found that chronic PYM treatment caused sperm shape abnormalities in Swiss-albino male mice in a recent study (unpublished data).

It is known that PYM is a suspected spindle poison (Yamamoto and Kikuchi, 1980) and this type of compound may lead to aneuploidy in somatic and germ cells. In germ cells especially, aneuploidy is important and may cause spontaneous abortions and infertility. If a germ cell carries aneuploid chromosomes it may abort in the early and late embriyonic stages.

Based on these studies, we suggest that PYM may be a germ cell mutagen *in vivo* and a possible genotoxic agent and further *in vivo* mutagenicity studies are needed.

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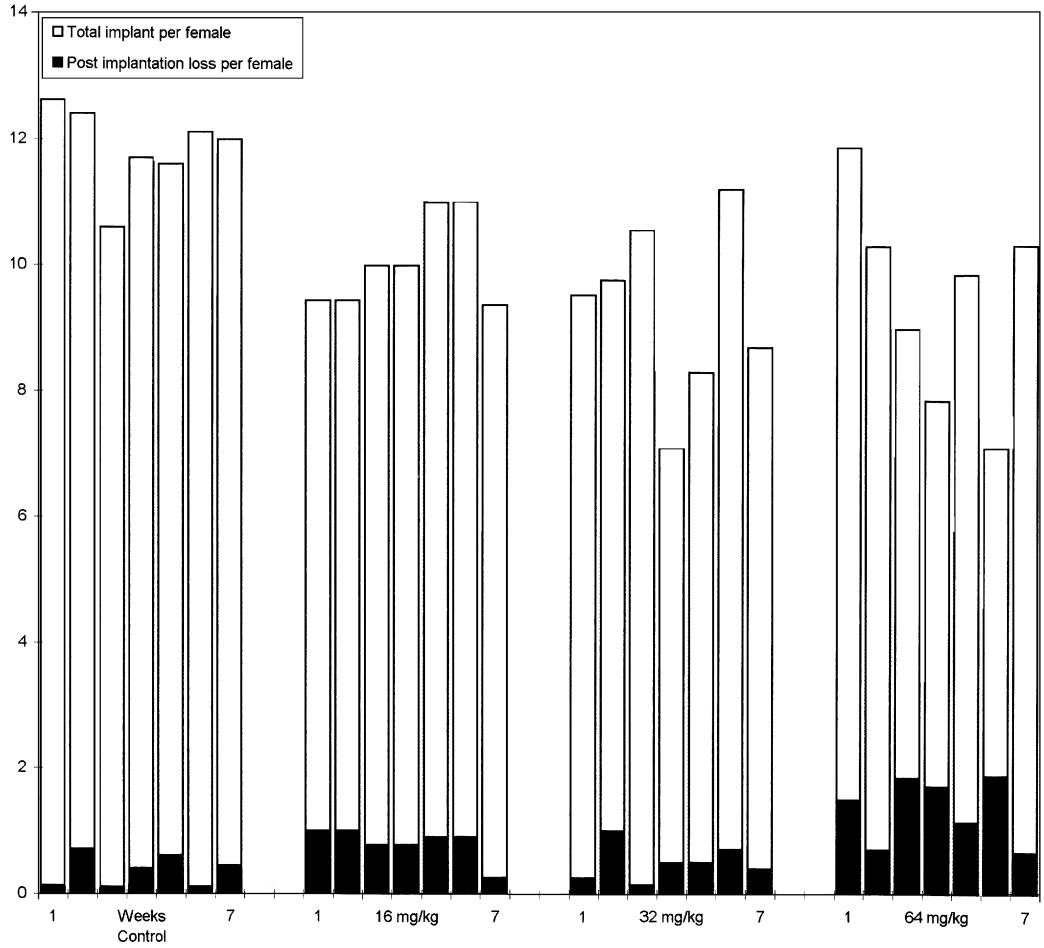

Fig. 1. Mouse dominant lethal mutation data for pyrimethamine. Matings were performed at weekly intervals for 7 weeks. Each column represents a 1 week interval.

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