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Cytogenetic Effect of Griseofulvin in Mouse Spermatocytes

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The genotoxic effects of griseofulvin (GF) in mouse primary spermatocytes at diakinesis metaphase I of meiosis were investigated. Griseofulvin was administered orally as a single dose of 500, 1000, 1500 and 2000 mg kg^{-1} body wt. and a multiple treatment with a daily dose of 1000 mg kg^{-1} body wt. for three and five successive doses. Both single and multiple treatment induced a statistically significant increase in the percentage of chromosomal aberrations which have a dose and time-dependent relationship. The frequency of chromosomal aberrations peaked 6 and 12 h post treatment; with the highest dose of the drug it reached 27.8% ± 0.87 and 27.66% ± 0.48 6 and 12 h respectively, compared with 5.6% ± 0.39 and 5.2% ± 0.48 for the control.

The types of aberrations recorded were structural, including X–Y and autosomal univalent, gaps, breaks, fragments, chain IV and numerical in the form of diploid, triploid, tetraploid and aneuploid.

The results of this study suggest that griseofulvin has a genotoxic effect in mouse spermatocytes.

INTRODUCTION

Attention has been focused recently on the biological and cytogenetic activity of drugs and chemicals.[1] Griseofulvin (GF) is an oral antifungal agent used for treating dermatophytosis by oral administration. It is active against dermatophytic fungi of different species in the genera *Microsporum*.[2,3] It can bind to microtubule-associated proteins and affect their incorporation into microtubules, thereby influencing the stability of the microtubules.[4]

The ability of griseofulvin to induce damage to the mitotic spindle and aneuploidy in various somatic cells has been reviewed.[5] Griseofulvin induced aneuploid mouse diakinesis metaphase II (MII) oocytes and ovulated diakinesis metaphase I (MI) oocytes, and their frequencies varied as a function of treatment time relative to ovulation when oocytes were harvested 18 h post treatment with human chorionic gonadotrophin (HCG).[6] It was also reported that GF-induced aneuploid MII oocytes can be fertilized and transmitted to zygotes.[7] This finding suggests genetic risk and agrees with earlier data involving colchicine.[8] Griseofulvin significantly increased the frequencies of oocytes blocked in MI and hyperploid MII oocytes.[9] Extensive work should therefore be conducted to identify the possible adverse genotoxic effects of griseofulvin. In the present investigation the induction of chromosomal aberrations in mouse spermatocytes was studied.

EXPERIMENTAL

Male Swiss mice of 9–12 weeks old were given griseofulvin in a 0.2-ml volume by oral gavage. Griseofulvin was obtained in the form of white powder from Mamfes Company of Chemicals and Drugs A.R.E. and before administration it was stirred in olive oil with a magnetic stirrer for 30 min. Controls were tested with vehicle.

Germ cell samples were taken: 24 h after a single oral dose of 500, 1000, 1500 or 2000 mg kg^{-1} body wt.; 6, 12, 24 and 48 h after a single dose of 2000 mg kg^{-1} body wt.; 24 h after the third treatment with a daily dose of 1000 mg kg^{-1}; and 24 h or 8 days after the fifth treatment with a daily dose of 1000 mg kg^{-1}.

In order to obtain 1 *ry* spermatocytes at MI, animals were intraperitoneally injected with colchicine (0.04 g kg^{-1} body wt.) 2.5 h before sacrificing. Testes were dissected out and processed according to Evans *et al.*[10] Slides were stained with 7% Giemsa in pH 6.8 phosphate buffer.

The effect of GF on meiosis was monitored by tracing chromosome damage in at least 50 diakinesis metaphase figures per animal. The number of animals used per treatment group ranged from four to seven, as shown in Tables 1–3. The significance of the experimental versus control data was calculated with a *t*-test.

RESULTS

The frequencies and distribution of chromosome rearrangements observed at MI of mice spermatocytes exposed orally to single and multiple doses of GF were recorded in Tables 1–3. The types of aberrations induced were mainly X–Y and autosomal univalent, gaps, breaks, fragments, chain IV and numerical aberrations in the form of diploid, triploid, tetraploid and aneuploid (n+). Cells with sticky chromosomes were observed in most treatments.

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Table 1. Number and percentage of the different types of chromosomal aberrations in primary spermatocytes of male mice after single oral treatment with different doses of griseofulvin

Treatment and doses	Time of harvest after the last treatment (h)	Number of mice	Total number of examined metaphases	No. of abnormal metaphases	Mean % abnormal metaphases ±SEM		No. and % of metaphases with different types of chromosomal aberration[a]									
							A-u	X-Yu	A-u + X-Yu	Gap or break in X chrom.	Chain IV	Break	Frag.	Numerical chromosome aberration: Dip.	Trip.	Aneup.
Control	24	4	350	21	6.0 ± 1.5	No.	4	7	–	1	–	–	1	8	–	–
						%	1.14	2	–	0.28	–	–	0.28	2.28	–	–
Griseofulvin																
500 mg kg^{-1} body wt.	24	5	350	40	11.43 ± 0.89[b]	No.	4	8	–	2	–	3	2	21	–	–
						%	1.14	2.28	–	0.57	–	0.86	0.57	6	–	–
1000 mg kg^{-1} body wt.	24	4	260	35	13.46 ± 0.36[b]	No.	4	9	–	3	1	–	–	16	2	–
						%	1.54	3.46	–	1.15	0.38	–	–	6.15	0.77	–
1500 mg kg^{-1} body wt.	24	5	250	53	21.2 ± 0.92[b]	No.	12	20	1	–	2	5	1	10	1	1
						%	4.8	8	0.4	–	0.8	2	0.4	4	0.4	0.4
2000 mg kg^{-1} body wt.	24	5	300	69	23.0 ± 0.6[b]	No.	5	15	–	6	2+1[c]	13	4	21	1	1
						%	1.66	5	–	2	1	4.3	1.3	7	0.33	0.33

[a]A-u = autosomal univalent; X-Yu = X–Y univalent; X chrom. = X chromosome; Frag. = fragments; Dip. = diploid; Trip. = triploid; Aneup. = aneuploid.
[b]Significant at the 0.01 level (*t*-test)
[c]Chain III + I.

Table 2. Number and percentage of the different types of chromosomal aberrations in primary spermatocytes of male mice at various harvest times after oral administration of griseofulvin, 2000 mg kg^{-1} body wt

Harvest time (h) post griseofulvin administration	Number of mice	Total number of examined metaphases	No. of abnormal metaphases	Mean % abnormal metaphases ±SEM		No. and % of metaphases with different types of chromosomal aberration[a]									
						A-u	X-Yu	A-u + X-Yu	Gap or break in X chrom.	Chain IV	Break	Frag.	Numerical chromosome aberration: Dip.	Trip.	Aneup.
6 hours															
Control	4	250	14	5.6 ± 0.39	No.	5	7	–	–	–	–	–	2	–	–
					%	2	2.8	–	–	–	–	–	0.8	–	–
Griseofulvin	6	510	142	27.8 ± 0.87[b]	No.	13	57	2	8	8	13	3	34	4	–
					%	2.5	11.18	0.39	1.57	1.57	2.55	0.59	6.6	0.78	–
12 hours															
Control	4	250	13	5.2 ± 0.48	No.	8	4	–	–	–	–	–	1	–	–
					%	3.2	1.6	–	–	–	–	–	0.4	–	–
Griseofulvin	6	300	83	27.66 ± 0.48[b]	No.	4	43	5	1	2	6	2	18	1	1
					%	1.3	14.3	1.66	0.33	0.66	2	0.66	6	0.33	0.33
24 hours															
Control	4	350	21	6.0 ± 1.5	No.	4	7	–	1	–	–	1	8	–	–
					%	1.14	2	–	0.28	–	–	0.28	2.28	–	–
Griseofulvin	5	300	69	23.0 ± 0.6[b]	No.	5	15	–	6	2+1[c]	13	4	21	1	1
					%	1.66	5	–	2	1	4.3	1.3	7	0.33	0.33
48 hours															
Control	4	260	13	5.0 ± 0.25	No.	2	5	–	–	–	–	–	6	–	–
					%	0.77	1.92	–	–	–	–	–	2.3	–	–
Griseofulvin	6	450	66	14.66 ± 0.57[b]	No.	1	10	2	4	5	5	5	31	2	1
					%	0.22	2.2	0.44	0.88	1.1	1.1	1.1	6.89	0.44	0.22

[a]For abbrevations, see legend to Table 1.
[b]Significant at 0.01 level (*t*-test).
[c]Chain III+I.

Table 3. Number and percentage of the different types of chromosomal aberrations in primary spermatocytes of male mice after multiple oral treatment with griseofulvin, 1000 mg kg^{-1} body wt

Treatment	Time of harvest after the last treatment (h)	Number of mice	Total number of examined metaphases	No. of abnormal metaphases	Mean % abnormal metaphases ±SEM		No. and % of metaphases with different types of chromosomal aberration[a]										
							A-u	X-Yu	A-u + X-Yu	Gap or break in X chrom.	Chain IV	Break	Frag.	Numerical chromosome aberration: Dip.	Trip.	Tetrap.	Aneup.
Three successive doses	24																
Control		4	260	16	6.15 ± 0.40	No.	2	7	–	–	–	–	–	7	–	–	–
						%	0.77	2.69	–	–	–	–	–	2.69	–	–	–
Griseofulvin		6	450	85	18.89 ± 1.0[b]	No.	14	18	1	2	3+1[c]	7	3	36	–	–	–
						%	3.1	4	0.22	0.44	0.88	1.55	0.66	8	–	–	–
Five successive doses	24																
Control		4	260	17	6.5 ± 0.63	No.	3	8	–	–	–	–	–	6	–	–	–
						%	1.15	3.08	–	–	–	–	–	2.3	–	–	–
Griseofulvin		7	430	110	25.58 ± 0.8[b]	No.	13	31	–	4	–	3	3	44	8	2	2
						%	3	7.2	–	0.93	–	0.7	0.7	10.2	1.86	0.46	0.46
Five successive doses and recovery	8 days																
Control		4	260	14	5.38 ± 0.29	No.	2	4	–	–	–	–	–	8	–	–	–
						%	0.77	1.54	–	–	–	–	–	3.07	–	–	–
Griseofulvin		5	300	57	19.0 ± 0.67[b]	No.	18	24	3	–	–	–	2	10	–	–	–
						%	6	8	1	–	–	–	0.66	3.3	–	–	–

[a]For abbreviations, see legend to Table 1.
[b]Significant at 0.01 level (*t*-test).
[c]Chain III + I.

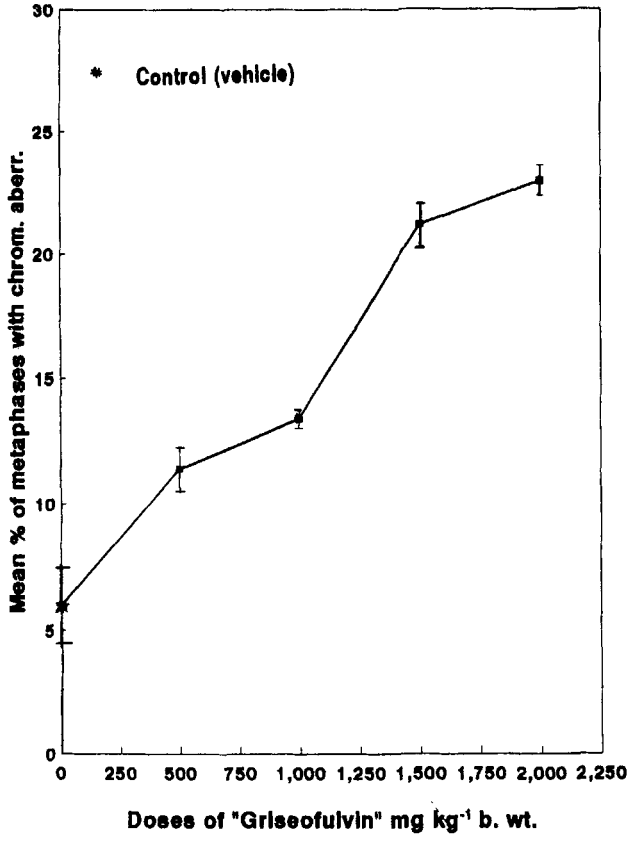

Figure 1. Percentage of metaphases with chromosomal aberrations in mouse spermatocytes 24 h after single oral treatment with different doses of Griseofulvin.

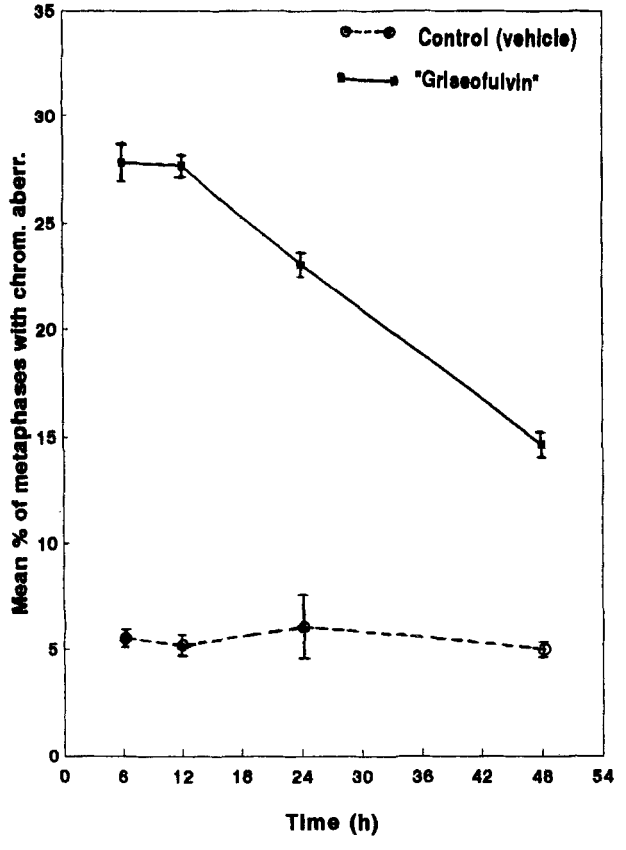

Figure 2. Percentage of metaphases with chromosomal aberrations in mouse spermatocytes after single oral treatment with griseofulvin 2000 mg kg^{-1} body wt.

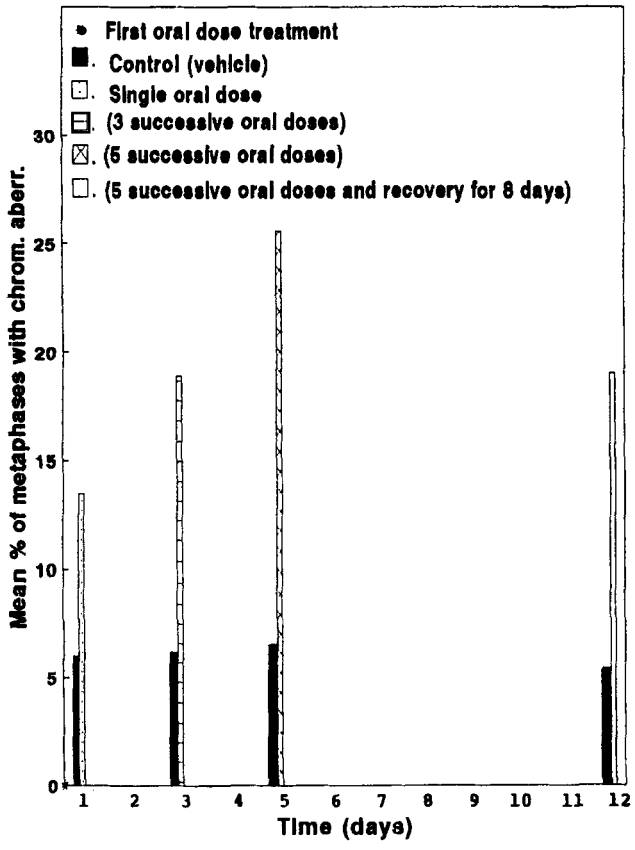

Figure 3. Percentage of metaphases with chromosomal aberrations in mouse spermatocytes after single and multiple oral treatment with griseofulvin, 1000 mg kg^{-1} body wt.

Single treatment

Oral treatment with a single dose of 500, 1000, 1500 and 2000 mg kg^{-1} body wt. induced statistically significant chromosomal aberrations in 1 *ry* spermatocytes 24 h after treatment. The result showed a dose–response relationship (Fig. 1) as the number of abnormal cells increased with increasing dose.

In the group of animals treated with 2000 mg kg^{-1} body wt., aberrations peaked at 6 and 12 h after treatment (Fig. 2).

Multiple treatment

Three and five daily doses of 1000 mg kg^{-1} body wt. increased the percentage of aberrant cells at 24 h more than a single dose. Even 8 days after treatment, this increase remained significant although less so than at 24 h (Fig. 3).

DISCUSSION

It is particularly relevant to study the genotoxic effects of various agents in germinal cells because this is the only system in which transmissible genetic damage from one generation to another takes place.[11]

In our study GF induced chromosome rearrangements after single and multiple treatment. It caused statistically significant increases ($P < 0.01$) in the number of aberrant 1 *ry* spermatocytes at all stages of the

investigation which were dose dependent. This is in accordance with De Carli and Larizza,[5] who reported that the extent of cytological damage generally depends on drug concentration, and they added that low GF doses may alter spindle orientation, resulting in partially affected metaphases and anaphases, whereas high doses lead to total disruption of mitotic microtubules.

Over time the opposite was true: the longer the time after treatment, the lower the frequency of the aberrant 1 *ry* spermatocytes. This also supports the opinion of De Carli and Larizza,[5] who reported that GF penetrates cells faster than colchicine and its effects on cells are reversed more rapidly upon removal of the drug. Also, GF has a half-life in plasma of about 1 day and approximately 50% of the oral doses can be detected in urine within 5 days.[3] Thus, for germ cells, as is known for many cell types, meiotic cell-cycle delay may be viewed as a protective mechanism.[12,13]

Multiple doses caused a higher increase in aberrant cells than a single dose. This may be due to the accumulation of the affected cells.

The increase in the aberrant cells 8 days after five successive daily doses was still significant although less so than after 24 h. According to Oakberg[14] these aberrant cells were at early prophase stages at the time when GF was administered, while those harvested 24 h after 3–5 days of treatment were at the pachytene stage. From the literature, there is no conclusive agreement upon which stage of prophase in spermatocytes is most sensitive to induction of chromosome aberrations.[11] According to our result we can conclude that early prophase stages are less sensitive than mid- or late prophase stages.

Chromosome translocation expressed in MI spermatocytes (rings and chains) has been observed in animals whose spermatogonia were treated with ionizing radiation [15–17] but rarely in those treated with chemicals.[18–22] In the present study, GF induced translocations in the form of chain IV which appeared in all doses given except the lower one (500 mg kg^{-1} body wt.) and in five successive doses harvested 24 h or 8 days after the last treatment. Its frequency is not high; it ranges from 0.38 to 1.57%. The higher percentage was recorded at the larger dose (2000 mg kg^{-1} body wt.) 6 h after treatment.

Polyploidy observed after treatment with GF suggests an effect on the meiotic spindle. The relationship of the spindle and the chromosomes may be disturbed to the extent that the spindle is aborted at some stage or is absent, which results in the duplication of the chromosome complement or polyploidy. This type of polyploidy is known as endopolyploidy and may be caused by different processes.[23,24] The disorganization of the meiotic spindle is not due to the dissolution of microtubules, but to the disorientation of intact microtubules. This effect seems to develop through an action on the poles of the spindle.[25]

The observed chromosome stickiness in our data is supported by the finding of Mailhes *et al.*[9] They reported that GF is responsible for damaging chromatin, resulting in chromosome clumbing/stickiness and a decreased percentage of MII oocytes. Chromosome stickiness may be an indication of cytotoxicity and should then be classified as non-transmissible, because the affected cell will either die or recover.

It is therefore possible to conclude from the present study that GF may be genotoxic and that it has a specific effect on the spindle.

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