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CYTOTOXIC EFFECTS OF GRISEOFULVIN ON HUMAN NORMAL CELLS *IN VITRO*

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Abstract

The effects of griseofulvin (GF) on morphological changes, cell growth, DNA- and RNA-synthesis, and chromosomal changes were studied using a human normal diploid fibroblast strain (WI-38) and peripheral blood lymphocytes from healthy donors. Morphological changes of WI-38 cells were not very prominent as a result of exposure to a high concentration (20 μg/ml) of GF for 3 days. Cell growth and the DNA-synthesis of WI-38 cells and lymphocytes were inhibited by the 3-day-treatment with 20 μg/ml of the drug, while RNA-synthesis was not inhibited by the same treatment. Chromosomal analysis showed that chromosomal abnormalities of structure as well as of number were induced in the lymphocytes exposed to GF for 3 days in concentrations of more than 0.2 μg/ml ($p < 0.05$). These observations suggest that GF has an effect on cellular DNA and that there is a possibility of the drug being a mutagenic and/or carcinogenic agent in humans.

INTRODUCTION

Since 1959, griseofulvin (GF) which was first isolated by Oxford et al in 1939[1] has been clinically used in the treatment of specific dermatophyte infections in various body sites. Since then, it has been reported that the GF induces hepatomas in mouse[2-4], chromosomal aberrations of human and rat cells[5,6], pathological morphological changes of animals[7], teratogenesis of rats[8], human leukemia[9], and that it promotes tumor development in mice treated with methylcholanthrene[10]. Larizza et al[11] described that heteroploid transformation of chromosomes was induced very frequently (45 to 75 %) with human diploid fibroblasts and normal lymphocytes by a single treatment with 40 μg/ml of GF or by a continuous treatment with 5 μg/ml of GF. Based on Larizza's results, GF is potentially harmful to humans, especially as an etiological agent of human cancers. We investigated cytological effects of GF in

detail using human normal cells *in vitro* to learn whether the drug had a possibility to be mutagenic and/or carcinogenic to human cells.

MATERIALS AND METHODS

Griseofulvin:

GF was kindly supplied by ICI-Pharma Ltd. (Osaka, Japan). The drug was dissolved in dimethylsulfoxide (DMSO) in a concentration of 5 mg per ml. This stock solution was diluted in desired concentrations with culture media before use. Our experiments were carried out in the concentration range of 0.1 to 20 μg/ml of GF, because mean serum concentrations *in vivo* are 1.4 to 1.72 μg/ml after an oral dose of 500 mg of GF is given daily.

Cell Culture:

Human diploid fibroblasts (WI-38)[12] were cultured in Eagle's Minimum Essential Medium (MEM) (Nissui Co., Tokyo) plus 10 % heat-inactivated fetal calf serum (FSC) (Flow Lab. Md.). Test tubes containing heparinized whole blood obtained from healthy donors were placed in a vertical position at 37°C for 2 to 3 hr and lymphocytes were collected from the supernatant plasma. The peripheral blood lymphocytes were cultured in RPMI 1640 Medium (Nissui Co., Tokyo) supplemented with 30% FCS and 0.2% phytohemagglutinin M (PHA M) (Gibco NY).

Toxicity Assay:

For morphological studies WI-38 cells grown on cover slips were stained with 2 % Giemsa after a 3-day-treatment with GF. In order to estimate the amount of survival in a cell population after the chimical treatment, WI-38 cells and lymphocytes were exposed to the drug for 3 days, stained with 0.1 % crystal violet in 0.1 M citric acid, and counted by means of a haemocytometer. Effects of GF on DNA- and RNA-synthesis of lymphocytes were studied by examining the incorporation of 1 μCi/ml ^{3}H-thymidine (5 Ci/mM, Radiochemical Centre, Amersham), or 1 μCi/ml ^{3}H-uridine (5 Ci/mM, Radiochemical Centre, Amersham) into 5 % cold trichloroacetic acid (TCA) insoluble cell fractions. ^{3}H-thymidine or ^{3}H-uridine was added for 30 min to the cultures after the cells were treated with GF for 3 days. All these experiments were performed in duplicate.

Karyology:

The lymphocytes treated for 3 days with 0.4 % DMSO, 0.2, 2.0, or 20 μg/ml of GF were submitted to chromosome analysis. Permanent slide preparations were prepared using conventional methods. One hun-

dred karyotypes were examined in each group and all abnormal karyotypes were photographed and analysed in detail.

RESULTS AND DISCUSSION

Morphological Studies :

WI-38 cells were used for these studies, because the cells which are human normal diploid cells and can grow on cover slips were suitable for morphological observations. Control cells which were not treated with GF grew and spread well on cover slips and showed a typical growth pattern of spindle-shaped fibroblasts. The cells treated with 0.4 % DMSO showed the same appearance as the control cells (Fig. 1). On the other hand, the cells treated with 20 μg/ml of GF for 3 days did not

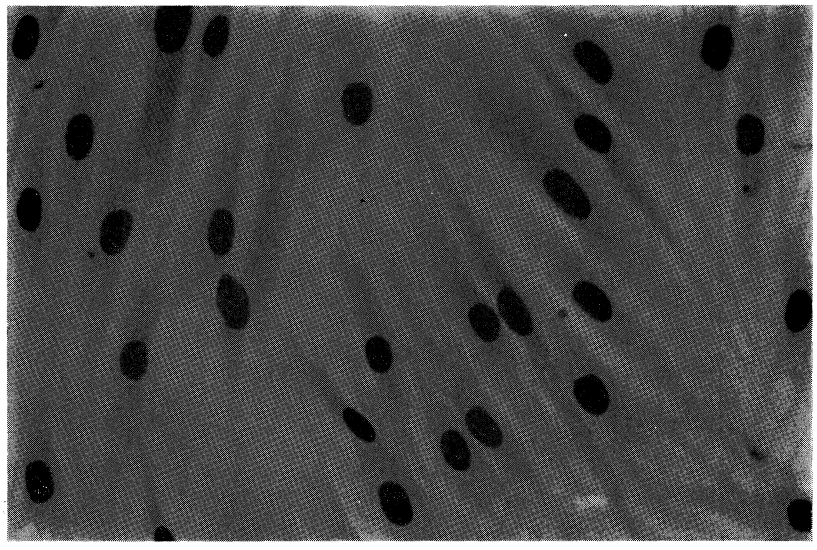

Fig. 1. WI-38 cells treated with 0.4 % DMSO shows a typical growth pattern of spindle-shaped fibroblasts. 10×20. Giemsa.

appear fibroblastic in shape but appeared epithelial-like. These cells with large polygonal cytoplasm contained many small vacuoles (Fig. 2). These morphological changes were not seen in the cultures as a result of 3-day-treatment with GF in concentrations of less than 10 μg/ml.

Cell Survival, DNA- and RNA-synthesis :

As shown in Table 1 and 2, the cell death of WI-38 cells and PHA-stimulated lymphocytes was not so prominent as a result of 3-day-treat-

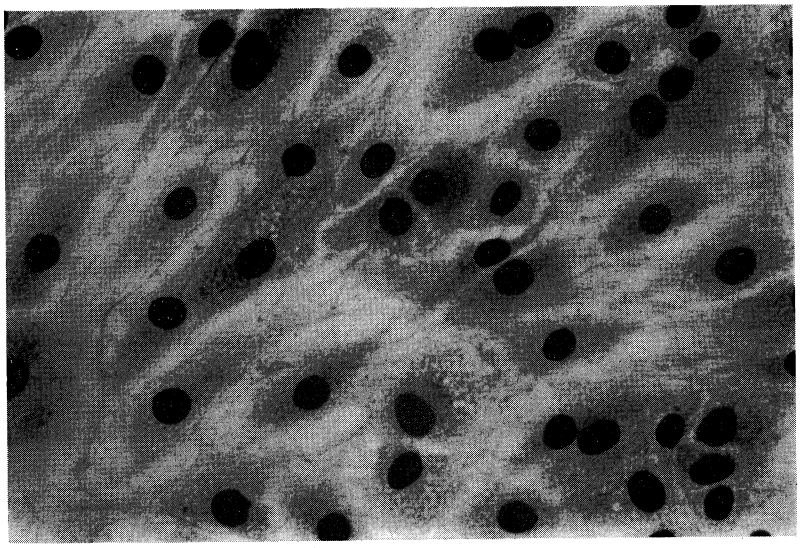

Fig. 2. WI-38 cells treated with 20 μg/ml GF for 3 days. The cells have large polygonal cytoplasm which contain many small vacuoles. 10×20. Giemsa.

TABLE 1.

Effects of griseofulvin (GF) on the multiplication and the DNA-synthesis of WI-38 cells

Conc. of GF (μg/ml)	No. of cells inoculated	No. of cells after treatment with GF	cpm/plate	cpm/10^6 cells	cpm of GF-treated culture / cpm of control culture (%)
0	250×10^3	550×10^3	948	2154	100
0.1	250×10^3	560×10^3	1386	3079	143
1.0	250×10^3	600×10^3	1237	2575	120
10	250×10^3	430×10^3	897	2638	122
20	250×10^3	390×10^3	588	1896	88

TABLE 2.

Effects of griseofulvin (GF) on the cell survival and the DNA-synihesis of PHA-stimulated human lymphocytes

Conc. of GF (μg/ml)	No. of cells inoculated per tube	No. of cells after treatment with GF for 3 days	cpm/10^6 cells	cpm of GF-treated culture / cpm of control culture (%)
0	10^6	960×10^3	6997	100
0.1	10^6	860×10^3	8742	125
1.0	10^6	850×10^3	8098	116
10	10^6	940×10^3	1917	27
20	10^6	720×10^3	1212	17

ment with 10 μg/ml of GF, but their DNA-synthesis was greatly inhibited by the same treatment. On the other hand, the rate of the RNA-synthesis of lymphocytes was not reduced by the GF-treatment (Table 3).

TABLE 3.

Effects of griseofulvin (GF) on the cell survival and the RNA-synthesis of PHA-stimulated human lymphocytes

Conc. of GF (μg/ml)	No. of cells inoculated	No. of cells after treatment with GF	cpm/tube	cpm/10^6 cells
0	680×10^3	760×10^3	5940	7815
0.1	680×10^3	750×10^3	8900	11867
1.0	680×10^3	720×10^3	6700	9306
10	680×10^3	700×10^3	6650	9500
20	680×10^3	620×10^3	5440	8774

Chromosme Analysis:

We carried out chromosome studies three times using lymphocytes obtained from three different donors. Fig. 3 shows one of these experiments. The lymphocytes exposed to 0.2 μg/ml of GF showed the highest frequency of aneuploidy (13 %) in the GF-treated experimental groups. The percentage of aneuploidy in the cultures treated with 2.0 and 20 μg/ml of GF was 8 % and 6 %, respectively. The decrease of aneuploidy in the cultures treated with more than 2.0 μg/ml of GF indicated that some cells damaged by GF could not proceed to a mitotic phase of cell cycle. All these aneuploid karyotypes observed were examined by photographic analysis. The loss of specific chromosomes could not be found in these cells. Structural abnormalities (gaps, breaks, dicentrics, deletion, translocation, etc.) of chromosomes were also examined. Seven to 11 % of GF-treated cells showed structural aberrations, while only 3 % cells had structural abnormalities in the control culture. Fig. 4 shows one example of chromosome aberrations seen in GF-treated lymphocytes. We did not find as many heteroploid transformations of human normal cells as Larizza[11].

The significant reductien of cell survival, the decreased DNA-synthesis, and chromosomal aberrations can not be related directly to mutagenicity and/or carcinogenicity of GF for humans at the present time, but the GF which can react with or damage chromosomes and DNA may be a potential mutagen and/or oncogenic substance. We have not

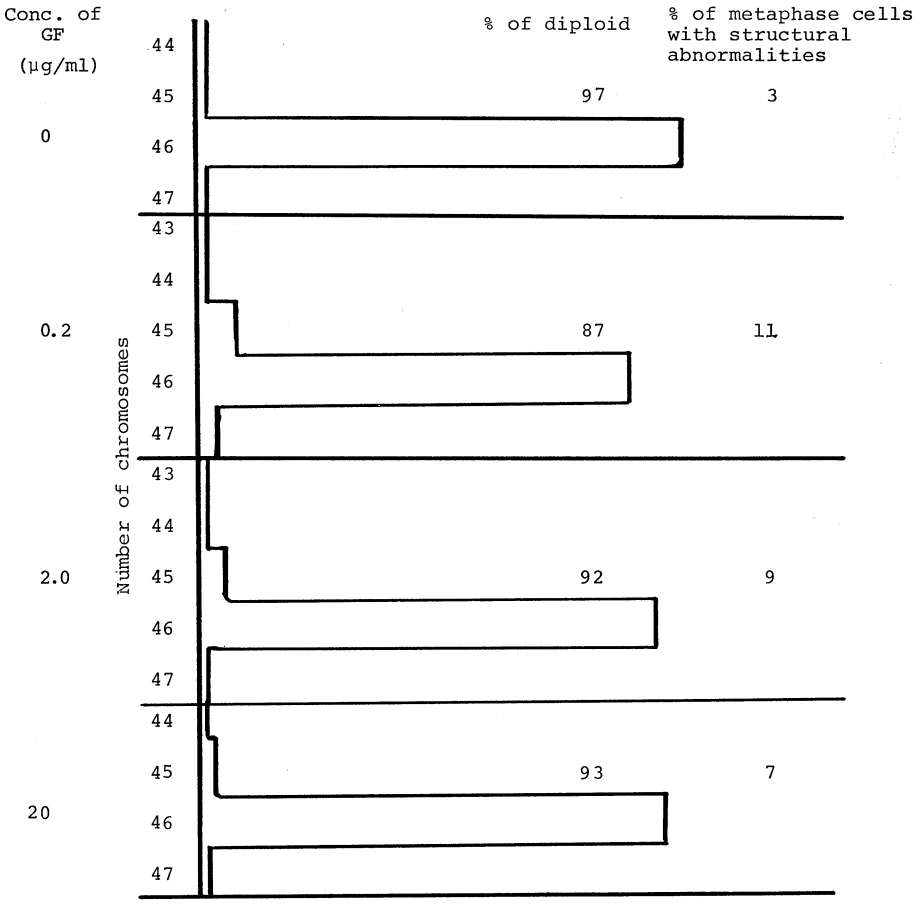

Fig. 3. Effects of griseofulvin on chromosomes of human lymphocytes.

yet studied the cytological effects of long-term treatment of human cells with GF but believe it should be done because GF is usually administered therapeutically for a relatively long period of time during which careful clinical observation on the toxicity of GF is required.

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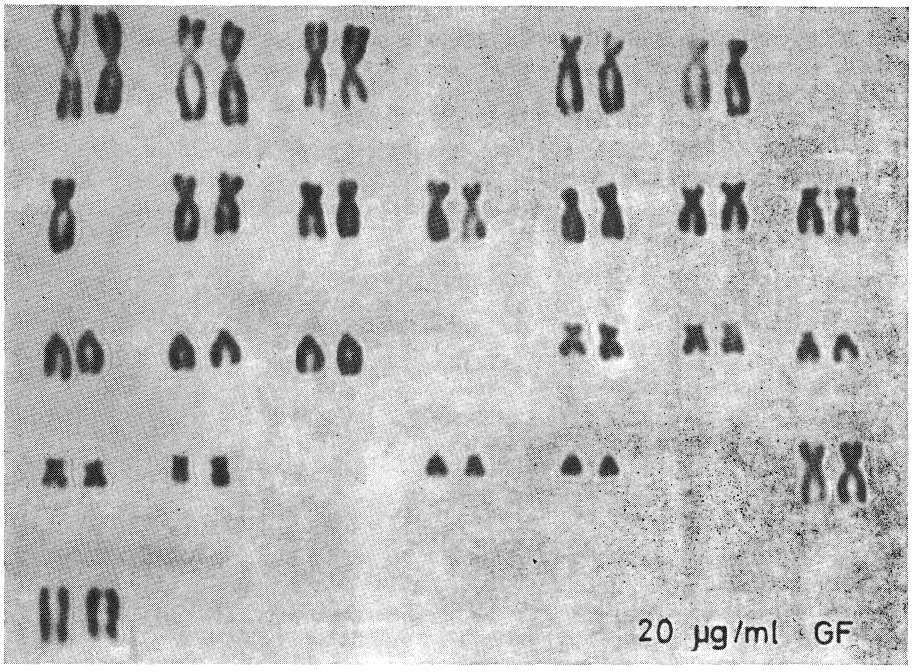

Fig. 4. One of examples of chromosome aberrations seen in griseofulvin treated cells. The absence of one chromosome from group C and the presence of two acentric fragments. 10×100. Giemsa staining.

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