Hepatocarcinogenicity of Griseofulvin following Parenteral Administration to Infant Mice

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SUMMARY

Random-bred infant Swiss mice were injected subcutaneously with suspensions of griseofulvin in tricaprylin according to various dosage schedules. Doses of griseofulvin in excess of 0.25 mg on Day 1 of life produced acute toxicity. Following a total dosage of 3.0 mg in infant mice, 0.5, 0.5, 1.0, and 1.0 mg at ages of 1, 7, 14, and 21 days, respectively, a high incidence of hepatomas, in 44% of 16 male mice alive at 49 weeks, developed in comparison with solvent controls, 8% of 48 males.

INTRODUCTION

Griseofulvin (7-chloro-4:6:2'-trimethoxy-6'-methylgris-2'-en-3:4'-dione) (Chart 1) is a potent fungal antibiotic commonly administered by prolonged oral dosage for therapy of veterinary and human dermatophytoses (1, 6). Large-scale clinical trials of Tinea capitis infections with this drug were recently conducted in children by WHO, and mass treatment was recommended in endemic areas (13, 23). The use of griseofulvin as an insecticide has been recently suggested (2).

Griseofulvin binds to nucleic acids and proteins of sensitive fungi (14), inhibits cuticle formation and morphogenesis in insects (2), produces metaphase delay and multipolar mitoses in Vicia faba root tips in vitro and mammalian cells in vivo (26), and potentiates colchicine toxicity in mice (19). Griseofulvin is demethylated by sensitive fungi (7, 14) and microsomal liver enzymes (5, 9, 11), and induces synthesis of δ-aminolevulinic acid synthetase, a rate-limiting enzyme in porphyrin metabolism, in primary tissue cultures of liver cells (22). At normal therapeutic levels of 10 mg/kg in man, griseofulvin increases fecal and erythrocyte protoporphyrin (27) and produces occasional acute toxicity (1). Oral dosage of rodents with griseofulvin, generally at the 1% level which corresponds to about 100 times human therapeutic dosage (3), produces growth inhibition, testicular atrophy (29), cocarcinogenicity (3), hypercholesterolemia, hepatic porphyria, hepatomegaly (11), hepatotoxicity, and hepatocarcinogenicity (4, 12, 24).

The hepatocarcinogenicity of griseofulvin, following parenteral administration of mg quantities to infant mice, is reported here.

MATERIALS AND METHODS

Suspensions of griseofulvin, at concentrations of 2.5, 5, and 50 mg/ml in redistilled tricaprylin, were stored in sealed ampoules at 4°C and used within one week of preparation. Using previously described technics (17,18), suspensions were injected subcutaneously in the nape of the neck of random-bred infant Swiss mice (ICR/Ha) at ages of 1, 7, 14, and 21 days in volumes of 0.1, 0.1, 0.2, and 0.2 ml, respectively; controls received solvent alone (Table 1). For practical considerations, all mice in each litter were treated alike. The solvent control group was the largest as this also served for other drugs concurrently tested. Although 2 litters were initially randomly assigned to each of the various treatment groups, the uneven number of litters finally assigned to the various groups reflected an attempt to concentrate testing at the highest possible subtoxic drug concentration (Table 1). This stepwise dose selection was accomplished over the span of a few days, during which mice were consecutively littering, so that all tests may be considered as simultaneous.

Following weaning and sexing, generally at 28 days, groups of 5 or fewer mice of each sex were housed in hanging metal cages with wire grid floors and given Purina breeder chow and water ad libitum. Mice were inspected daily and weighed weekly for the first month of life and at approximately monthly intervals thereafter. Mice were allowed to survive, with the exception of those sacrificed when moribund, until experiments were terminated between 49 and 51 weeks (49±). With occasional exceptions due to autolysis, cannibalism, or accidental loss, all mice were autopsied and tissues from any lesion or tumor and usually also from liver, spleen, heart, lungs, kidneys, adrenals, thymus, lymph glands, and seminal maw were fixed in Tel-lyeneichky fluid, sectioned at 5 μ, and stained with hematoxylin and eosin.

RESULTS

Mortality before weaning (Table 1) was comparable in solvent controls (14%) and in neonates receiving 0.25 mg of griseofulvin on Day 1 (12%); higher mortality was, however, observed with 0.5- and 5.0-mg doses on Day 1 (42% and 100%, respectively). Death before weaning in all groups was largely limited to the first few days of life, especially following initial drug doses of 0.5 and 50 mg, so that mortality differences between different groups also occurred then. Following weaning, substantial sex differences in survival generally developed due...
to a nonspecific syndrome of obstructive uropathy in male mice (16); the incidence of uropathy, based on numbers of males alive at weaning and subsequently autopsied, was high in all groups: controls, 38%; Groups I and II griseofulvin, 46% and 24%, respectively. Relative to controls, there was no evidence of weight loss at any time in drug-treated mice.

A markedly enhanced incidence of hepatomata was noted at termination of experiments in male mice receiving 0.5 mg of griseofulvin on Day 1 as compared with solvent controls; the corresponding percentage incidences of hepatomata, based on males at risk at 49+ weeks, are 44% (Group II) versus 8% (controls), respectively (Table 2). The incidence of hepatomata (18%) in mice receiving 0.25 mg of griseofulvin on Day 1 (Group I) occupies an intermediate rank between corresponding values for the solvent controls and the group receiving a corresponding initial dosage of 0.5 mg; the figures for the lower dosage group, however, are based on smaller numbers. No instances of spontaneous or induced hepatomata occurred in female mice in these experiments.

An apparent litter influence on the distribution of hepatomata was noted. For example, in Group II at 49+ weeks, a total of 7 hepatomata were distributed among 16 male mice deriving from 6 different litters as follows: 1/1, 0/2, 0/3, 2/3, 3/3, 1/4, where for each individual litter, the numerator and denominator represents the number of males with tumor and the total number of surviving males, respectively (Table 2). An analysis of variance of the various tumor yields in Group II and controls (44% versus 8%), performed so as to take any possible litter differences into account, yields, $F(1, 16) = 6.23, P < 0.025$. Ignoring any possible litter effect, conventional analysis would have yielded even

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**TABLE 1**

Acute Toxicity Induced in Swiss Mice by Neonatal and Perinatal Subcutaneous Injections of Suspensions of Griseofulvin in Tricaprylin

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Drug dosage on specified days</th>
<th>Total drug dosage (mg)</th>
<th>Initial No. of males injected</th>
<th>No. of litters</th>
<th>Av. weight (gm) of mice at specified days</th>
<th>% mortality prior to weaning</th>
<th>Sex</th>
<th>No. of survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td>At weaning</td>
</tr>
<tr>
<td>Tricaprylin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Griseofulvin (Group I)</td>
<td>0.25</td>
<td>0.50</td>
<td>1.00</td>
<td>1.00</td>
<td>2.75</td>
<td>26</td>
<td>1.7</td>
<td>12.3</td>
</tr>
<tr>
<td>Griseofulvin (Group II)</td>
<td>0.50</td>
<td>0.50</td>
<td>1.00</td>
<td>1.00</td>
<td>3.00</td>
<td>92</td>
<td>1.8</td>
<td>14.0</td>
</tr>
<tr>
<td>Griseofulvin (Group III)</td>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
<td>5.00</td>
<td>43</td>
<td>1.5</td>
<td>100</td>
</tr>
</tbody>
</table>

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**TABLE 2**

Hepatomata and Other Hepatic Lesions Developing in Male Swiss Mice following Neonatal and Perinatal Injections of Tricaprylin or Suspensions of Griseofulvin in Tricaprylin

Results are shown separately for each litter in the study.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Initial drug dosage on Day 1 (mg)</th>
<th>No. males at risk at 49+ weeks</th>
<th>Individual litter results* for surviving males at 49+ weeks (No. male mice with specified hepatic lesions/No. male survivors in each litter)</th>
<th>Hepatic lesions (incidence as % of males alive at 49+ weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moderate atypia</td>
<td>Advanced atypia</td>
</tr>
<tr>
<td>Tricaprylin</td>
<td></td>
<td></td>
<td>0/0, 0, 0/0, 0/0, 0/0, 0/1, 0/2, 0/2, 0/3, 0/3, 0/4, 0/4, 0/5, 0/5, 1/5, 0/7, 0/7.</td>
<td>0/0, 0, 0/0, 0/0, 0/0, 0/1, 0/2, 0/2, 0/3, 0/3, 0/4, 0/4, 0/5, 0/5, 0/7, 0/7.</td>
</tr>
<tr>
<td>controls</td>
<td></td>
<td></td>
<td>0/5, 0/5, 0/6.</td>
<td>0/5, 0/5, 0/5, 0/5, 0/7.</td>
</tr>
<tr>
<td>Griseofulvin (Group I)</td>
<td>0.25</td>
<td>11</td>
<td>0/0, 0/1, 0/2, 0/3, 0/3, 1/3, 0/4.</td>
<td>0/0, 0, 0/0, 0/0, 0/1, 0/2, 0/2, 0/3, 1/3, 0/4.</td>
</tr>
</tbody>
</table>

* Data for each litter occupy the same position in each column.

Moderate and advanced hepatic atypia correspond to MHA and AHA, respectively, in Table 3.
Separately for male and female survivors following weaning, the table shows the following data: (a) If the animal died during the study period (49-51 weeks), it shows whether with or without a tumor, the week of death, and what tumors or other lesions were found at autopsy. Accidental losses and autolyzed animals are identified. Where more than one animal have identical data, both on week of death and tumors or lesions, this is indicated by the symbol X followed by the number of identical observations, (b) If the animal survived the study period, and was then sacrificed, the table shows whether a tumor was found at autopsy, and identifies any additional lesions which may have been revealed. The symbol X followed by a number indicates instances of identical tumor and lesion data for terminally sacrificed animals.

**TABLE 3**

**Survival, Tumors, and Other Lesions Developing in Swiss Mice Injected Initially as Neonates with Tricaprylin or Tricaprylin Suspensions of Griseofulvin**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex and Nos. at weaning</th>
<th>Mice dying or sacrificed during course of experiments</th>
<th>Mice sacrificed at conclusion of experiments (49-51 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Deaths with tumor</td>
<td>Deaths without tumor and losses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>Individual week of death and associated tumors or lesions</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>Griseofulvin, Group I</td>
<td>M</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Griseofulvin, Group II</td>
<td>M</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Griseofulvin, Group III</td>
<td>M</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Parentheses are used to indicate instances where no autopsy was possible due to autolysis (A) or accidental loss (L). Code for tumors or lesions at death: Tumors: H, hepatoma; MC, mammary carcinoma; PA, pulmonary adenoma; TL, thymic lymphoma. Other lesions: AHA, advanced hepatic “atypia”; B, bronchopneumonia; LY, lymphadenitis (nonspecific); M, myocarditis; MHA, moderate hepatic “atypia”; N, nephropathy; PN, pyelonephritis; T, trauma; U, obstructive uropathy.

More significant differences, continuity-corrected chi square = 8.23, P < 0.005.

The hepatomata were solitary or multiple and varied in size from the microscopic to that exceeding the normal mouse liver (Fig 1). Generally, the tumors were of the classic, well-differentiated and solid hepatocellular or trabecular type, with conspicuous hyaline and refractile eosinophilic cytoplasmic inclusion bodies (Figs 2, 3); occasionally dilated sinusoids were prominent with a consequent hemangiomatous or alveolar pattern (Fig. 4). No extrahepatic metastases were noted. The high incidence of hepatomata in Group II griseofulvin was associated with atypia in the livers of nontumor-bearing male mice. These atypia, classified in Table 2 as moderate and advanced, depending on their extent and severity, featured focal or lobular disruption of normal hepatic architecture and were generally associated with areas of histiocytic infiltration, zonal necroses, nuclear and cytoplasmic inclusion bodies, increased cytoplasmic basophilia and cellular pleomorphism (Figs. 5, 6). If the advanced atypia, as possible preneoplastic lesions, are grouped with the hepatomata, so that the comparison is 63% versus 8% (Table 2), then differences between Group II and controls are further emphasized, continuity-corrected chi square = 17.55 P < 0.0001; taking litter effect into account, F(1, 16) = 15.32, P < 0.002.

Further details of the results of this experiment, excluding individual litter information, are given in Table 3. As can be seen, a few other tumors were noted: male controls, 3 solitary pulmonary adenomata, 1 histiocytic malignant lymphoma; female controls, 2 solitary pulmonary adenomata, 1 mammary adenocarcinoma; griseofulvin Group I males, 3 solitary pulmonary adenomata, 2 of which were associated with hepatoma; Group I females, 2 solitary pulmonary adenomata; griseofulvin Group II males, 2 solitary pulmonary adenomata, 1 lymphocytic malignant...
lymphoma; Group II females, 1 solitary pulmonary adenoma together with a mammary adenocarcinoma. No local tumors at injection sites were noted in any animals.

DISCUSSION

In toxicity and carcinogenicity testing with neonates, it is necessary, for practical considerations, to treat all animals in each litter alike. This imposes limitations on the statistical consideration of all animals as individuals, without reference to possible litter influences (N. Mantel and S. S. Epstein, unpublished data). In the present experiments, the hepatocarcinogenicity of griseofulvin for male mice is highly significant, although the level of significance is slightly reduced when litter effect is taken into account.

Sex differences in the metabolism of griseofulvin in the rat (8) and the marked susceptibility of adult male Swiss mice to the hepatocarcinogenic effects of this drug (12) are in accord with the exclusive occurrence of hepatomas in males in the present experiments. Additionally, various other hepatocarcinogens also predominantly affect male mice following neonatal administration (17, 18, 28). The initial dosage of griseofulvin on Day 1 seems important as the incidence of hepatomas is higher in Group II (44%); with initial and total dosages of 0.5 and 3.0 mg, respectively, than in Group I (15%), with initial and total dosages of 0.25 and 2.75 mg, respectively. The 3.0-mg total dosage in Group II is equivalent to 200 mg/kg expressed relative to an approximate body weight of 15 gm on Day 21; the individual doses employed are equivalent to 250 mg/kg, 100 mg/kg, 100 mg/kg, and 67 mg/kg on Days 1, 7, 14, and 21, if the corresponding approximate animal weights are taken as 2, 5, 10, and 15 gm, respectively. The human therapeutic oral dosage of griseofulvin is in the order of 10 mg/kg daily over prolonged periods; this level maintained for 6 months represents a total dosage of 1800 mg/kg. Such dosages attain or greatly exceed levels found here to be highly carcinogenic. Further, many drugs are, on an mg/kg basis, more toxic in man than in the mouse, generally by a factor of 10–15 (21).

Various possibly offsetting factors must be noted. Therapeutically, griseofulvin is administered orally in aqueous suspension, not parenterally in an oily vehicle, and to children or adults, rather than to infant rodents as in the present experiments. If loss in drug activity due to use of the oral route is as great as 90%, this would compensate for the 10–15 mouse-to-man factor; however, although gastrointestinal absorption of griseofulvin in rodents and man is irregular and partial (20, 25), only approximately 16% of a single oral dose can be recovered from human feces (1). The efficiency of absorption in man has recently been increased by the use of micronized formulations (1, 25). Additionally, while the susceptibility of rodents to the acute toxic effects of the drug and its subsequent carcinogenicity seems maximal following neonatal parenteral administration, nevertheless previous data clearly show that neither age, suspending vehicle, nor route are critical (4, 12, 24). It is of interest to note that although enhanced neonatal susceptibility to drug toxicity, in general, probably reflects immaturity of hepatic drug-metabolizing enzymes (10), griseofulvin itself induces microsomal demethylases (5, 9, 11); inhibition of these induced enzymes probably accounts for the marked enhancement of acute toxicity noted in neonatal mice following combined administration of piperonyl butoxide, a microsomal enzyme inhibitor (10), together with griseofulvin (15).

The hepatocarcinogenicity of griseofulvin for male mice, following parenteral administration of mg quantities in infancy, is striking. These data thus complement previous evidence (12, 24) on the carcinogenicity of this drug following prolonged feeding of adult rodents at relatively high dosages. While it is difficult to extrapolate meaningfully from rodent to man, the present data pose the question as to whether the therapeutic utility of griseofulvin is such as to offset its possible carcinogenic hazard. These data further suggest consideration of the need for prospective epidemiologic investigations following human therapy, particularly of children, with griseofulvin.

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FIGS. 1-6. Sections of liver from male Swiss mice injected perinatally with 3.0 mg of griseofulvin and sacrificed at 50 weeks. H & E.

Fig. 1. Large hepatomas in right lateral and median lobes of liver.

Fig. 2. Trabecular hepatoma from right lateral lobe of liver in Fig. 1, with margin of compressed normal liver cells at upper left hand corner. X 115.

Fig. 3. Higher magnification of Fig. 2, illustrating hepatoma cells with multiple hyaline cytoplasmic inclusion bodies of varying sizes. X 400.

Fig. 4. Hemangiomatous hepatoma with prominent and dilated sinusoids. X 125.

Fig. 5. Advanced "atypia" featuring disruption of normal hepatic architecture, focal necrosis, marked cytoplasmic vacuolization, cellular and nuclear pleomorphism. X 240.

Fig. 6. Higher magnification of Fig. 5, illustrating nuclear enlargement and deformity and nuclear inclusion bodies. X 1200.

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