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AN EFFECT OF PHENOBARBITONE ON GRISEOFULVIN METABOLISM IN THE RAT

BY

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Prior administration of phenobarbitone to male and female rats dosed orally or intravenously with griseofulvin caused a fall in blood levels of the antibiotic. The effect of a single oral dose of phenobarbitone was significant after 12 hr and maximal between 12 and 48 hr, and it lasted for at least 96 hr ; it was more pronounced when the barbiturate was administered repeatedly. Liver slices from animals dosed with phenobarbitone metabolized griseofulvin more rapidly than did those from undosed animals. The possible relevance of these findings to the clinical use of griseofulvin is discussed.

Many substituted aromatic ethers are metabolized by oxidative dealkylation (Axelrod, 1955, 1956 ; Bray, Craddock & Thorpe, 1955 ; Davison, Wangler & Smith, 1962). Axelrod (1955, 1956) showed that such reactions take place in the liver, are catalysed by microsomal enzyme systems and result in the formation of aldehydes. Activators of these enzyme systems include phenylbutazone, orphenadrine and certain barbiturates, for example, phenobarbitone (Conney, Davison, Gastel & Burns, 1960). The antifungal antibiotic griseofulvin is an aromatic ether, and it is dealkylated by rat liver slices (Bedford, Busfield, Child, MacGregor, Sutherland & Tomich, 1960) to give formaldehyde (Basil, personal communication) and 6-demethylgriseofulvin (Barnes & Boothroyd, 1961), which has been isolated also from the urine of rats, rabbits and men dosed orally with the antibiotic (Barnes & Boothroyd, 1961). Because dermatologists often prescribe phenobarbitone concomitantly with griseofulvin, we thought it worth while to investigate whether the sedative could affect the metabolism of the antibiotic.

METHODS

Male and female rats of the PVG strain (body weights between 100 and 170 g) were used. Phenobarbitone, as the sodium salt, was administered orally in aqueous solution, and griseofulvin orally as a 5% suspension in 0.5% Tween 80 or intravenously as a 2% solution in 75% *NN*-dimethylformamide.

Blood samples (2 to 3 ml.) were taken by cardiac puncture from anaesthetized rats, with heparin (50 U in 0.1 ml. of 0.9% saline) added to the samples to prevent clotting. Each rat was bled once only.

Approximately 200 mg of rat liver slices were incubated at 37° C with 10 ml. of Krebs solution (NaCl 6.9, KCl 0.35, $CaCl_2$ 0.28, KH_2PO_4 0.16, $MgSO_4$ $7H_2O$ 0.3 and $NaHCO_3$ 2.1 g/l.)

containing griseofulvin (10 μg/ml.); 5% carbon dioxide in oxygen was bubbled through the solution during incubation. After 2 hr, 1 ml. volumes of the incubation fluid were assayed for griseofulvin.

The concentration of griseofulvin in the blood samples and in the solutions from the liver incubation experiments were determined by a spectrophotofluorimetric method (Bedford, Child & Tomich, 1959); we find that this method is unaffected by phenobarbitone or its metabolites.

RESULTS

Effect of single doses of phenobarbitone on blood levels of griseofulvin

Female rats, some of which had been dosed orally 24 hr before with phenobarbitone, received single oral doses of griseofulvin (100 mg/kg), and blood antibiotic levels were measured 4 hr later (Table 1). In a dose of 1.88 mg/kg the barbiturate

TABLE 1

EFFECT OF PHENOBARBITONE ON BLOOD GRISEOFULVIN LEVELS IN FEMALE RATS

Phenobarbitone sodium was administered orally 24 hr before griseofulvin. Blood griseofulvin levels (means and standard errors) were determined 4 hr after oral administration of 100 mg/kg

Dose of phenobarbitone sodium (mg/kg)	No. of rats	Blood griseofulvin level (μg/ml.)
0	35	3·7±0·2
1·88	15	3·0±0·2
3·75	18	1·9±0·1
7·50	18	2·0±0·1
15·00	18	1·6±0·1
30·00	18	1·8±0·1
60·00	18	1·6±0·1

only slightly reduced blood griseofulvin concentration, but the effect of 3.75 mg/kg was highly significant, and higher doses produced no greater effect. The highest dose of phenobarbitone not producing apparent sedation in the rats was 15 mg/kg, and this dose was therefore used in the experiments now to be described.

Griseofulvin (100 mg/kg) was administered orally to female rats 12, 24, 48, 72 or 96 hr after they had received phenobarbitone, and blood antibiotic levels were measured 4 hr later (Fig. 1). The effect was significant 12 hr after the administration of phenobarbitone, maximal between 12 and 48 hr and insignificant by 96 hr.

Curves relating times and blood concentrations of griseofulvin for both control and phenobarbitone-dosed male and female rats are shown in Fig. 2. Levels were higher in the females than in the males, and the levels in the phenobarbitone-dosed animals were lower and of shorter duration in both sexes than in the controls.

Effect of repeated doses of phenobarbitone on blood griseofulvin levels

Twenty-four female rats each received an oral dose of phenobarbitone (15 mg/kg) once daily for up to 14 days, and a similar group of untreated rats served as controls. On days 4, 8, 15 and 18 six rats from each group were dosed orally with griseofulvin

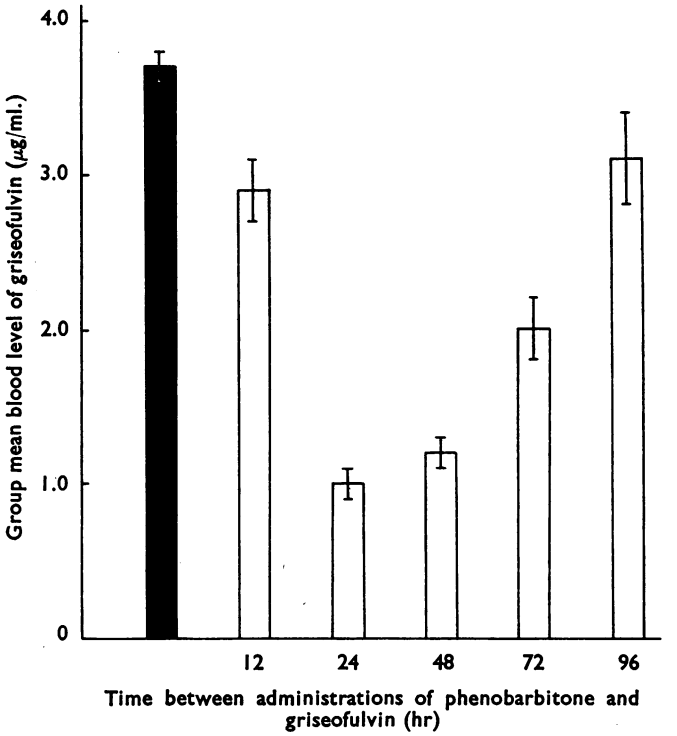

Fig. 1. Effect of phenobarbitone on blood levels of griseofulvin in female rats. Phenobarbitone sodium (15 mg/kg) was administered orally at various times before oral administration of griseofulvin (100 mg/kg). Blood antibiotic levels were measured 4 hr later. Filled column, control rats (n=23); Empty columns, phenobarbitone-dosed rats (groups of six). Vertical lines indicate standard errors.

(100 mg/kg), and blood antibiotic levels were determined after 4 hr. Phenobarbitone was not administered on the day of the blood test. The blood levels were considerably lower in the phenobarbitone-dosed rats than in the control animals (Fig. 3), and the effect of repeated dosing with phenobarbitone was greater than that of a single dose (Table 1). The blood levels in the animals receiving phenobarbitone were similar on days 4, 8 and 15, whereas the levels in the controls increased with the age of the animals. On the 4th day after the last dose of phenobarbitone there was no significant difference between the blood levels in the test and control animals.

Effect of phenobarbitone on blood antibiotic levels in rats injected intravenously with griseofulvin

Blood griseofulvin levels were determined in male and female rats 2 hr after they had received single intravenous doses of griseofulvin (20 mg/kg). Phenobarbitone (15 mg/kg) was administered orally to one group of six male rats and to one group of six female rats 24 hr before the griseofulvin. In both sexes the levels in the

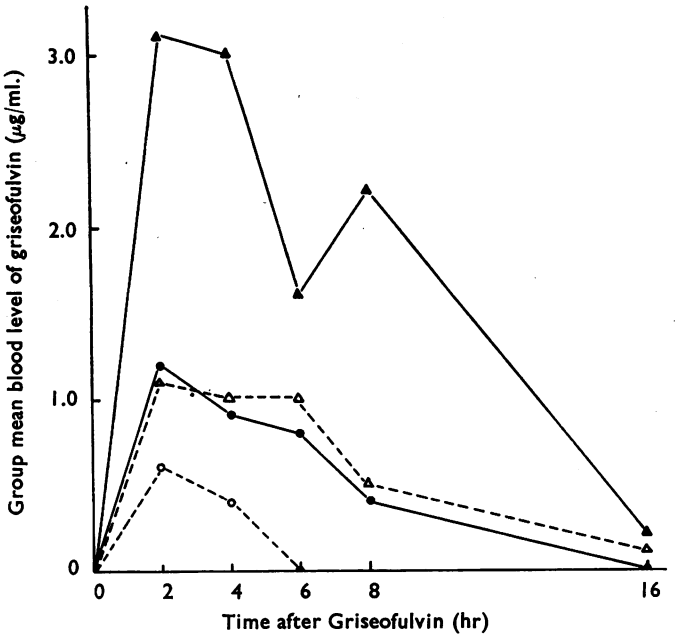

Fig. 2. Effect of phenobarbitone on blood levels of griseofulvin in male and female rats. Phenobarbitone sodium (15 mg/kg) was administered orally 24 hr before oral administration of griseofulvin (100 mg/kg). Blood antibiotic levels were measured 2, 4, 6, 8 or 16 hr later. ▲——▲, Control females (n=12); △---△, phenobarbitone-dosed females (n=6); ●——●, control males (n=12); and ○---○, phenobarbitone-dosed males (n=12). Standard errors are between 0.3 and 0.1.

phenobarbitone-dosed animals were lower than those in the controls, the group mean values being 0.2 and 0.5 μg/ml. for the males, and 1.7 and 3.7 μg/ml. for the females.

In vitro *metabolic activities of the livers of normal and phenobarbitone-dosed rats*

Rats were dosed orally with phenobarbitone (15 mg/kg) once daily for 3 days, and 24 hr after the last dose the animals were killed and their livers removed. The

TABLE 2

THE EFFECT OF PHENOBARBITONE ON THE METABOLISM OF GRISEOFULVIN BY LIVER SLICES

Phenobarbitone sodium (15 mg/kg) was administered orally 72, 48 and 24 hr before removal of the livers. The griseofulvin metabolized (means and standard errors) is given as μg/200 mg of liver slices/2 hr. The number of animals tested is given in brackets. The differences between sexes, and between treated and untreated groups, are all highly significant ($P<0.001$)

Previous treatment	Griseofulvin metabolized (μg/200 mg/2 hr)	
	Females	Males
None	48·3±1·2 (12)	59·5±1·1 (12)
Phenobarbitone	59·5±1·6 (6)	71·2±1·9 (6)

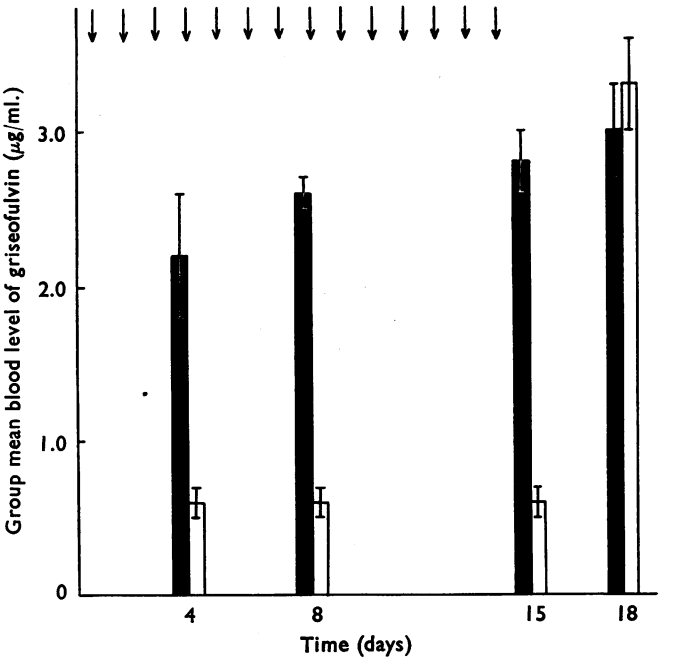

Fig. 3. Effects of repeated doses of phenobarbitone on blood levels of griseofulvin in female rats. Arrows indicate oral administrations of phenobarbitone sodium (15 mg/kg). Blood antibiotic levels were measured 4 hr after oral administration of griseofulvin (100 mg/kg). Filled columns, control rats; empty columns, phenobarbitone-dosed rats: six rats per group. Vertical lines represent standard errors.

amounts of griseofulvin metabolized in 2 hr by 200 mg of liver slices from dosed and undosed animals are given in Table 2. As reported earlier, the livers of male rats metabolized more griseofulvin than those of females (Busfield, Child, Basil & Tomich, 1960); the livers of phenobarbitone-dosed rats were more active than those of the controls. The sex difference was apparent in both control and phenobarbitone-dosed rats. The differences between the sexes and those between treated and untreated animals were highly significant ($P<0.001$).

DISCUSSION

The results of the incubation experiments suggest that the low blood griseofulvin levels in rats previously dosed with phenobarbitone reflect an increased rate of griseofulvin metabolism rather than an impairment of alimentary absorption. This view is supported by the low blood levels in rats dosed orally with phenobarbitone and intravenously with griseofulvin.

Most workers investigating enzmye activation administer the activating drug intraperitoneally, but in this series of experiments the oral route was used, to simulate clinical practice. It is not uncommon for patients taking griseofulvin for the treatment of skin and nail infections to receive also phenobarbitone in a total

daily dose of 1 to 1.5 mg/kg. Slightly larger doses of phenobarbitone produced an increase in the metabolism of griseofulvin in the rat; it is therefore conceivable that the administration of phenobarbitone to patients receiving griseofulvin could reduce its efficacy as an antifungal agent. The results of an experiment on volunteers indicates that low blood levels of this antibiotic are obtained after treatment with phenobarbitone (Busfield, Child, Atkinson & Tomich, 1963); these experiments are being continued.

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