In summary, our present observations indicate that the patterns of the age-associated alterations in the activities of *N*-demethylation and 2-hydroxylation of IMI are quite different in male rats, and that the former showed a marked sex difference while the latter did not. Therefore, present results are consistent with the hypothesis previously proposed by ourselves that these pathways are predominantly mediated by different species of cytochrome P-450 [1].

The fact that age-associated alteration in IMI metabolism is position selective together with our previous observations on the position selective alteration of lidocaine metabolism [7], provides strong supportive evidence for the hypothesis that age-associated alterations in drug metabolizing enzyme activities are caused by alterations in the relative abundance of cytochrome P-450 species with age [2, 3]. These observations may also have important clinical and toxicological implications, because the ratio of the amounts of active metabolite, DMI and a suspected toxic metabolite 2 OH-IMI, may be different depending on age and sex.

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Effects of griseofulvin on enzymes associated with Phase I and II of drug metabolism

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Griseofulvin (GF), an anti-fungal agent, has been used rather effectively in man. Although this compound exhibits potent antibiotic properties, a number of side effects, particularly in liver, have been reported. In mice, GF feeding markedly alters porphyrin metabolism [1]. Enlargement and darkening of the liver and histologic evidence of porphyrinstasis and cholestasis have been reported [2, 3]. Studies by DeMatteis and Gibbs [4] reported a decrease in hepatic ferro-chelatase of mice and rats treated with GF when measured by Co^{2+}-mesoporphyrin formation in isolated mitochondria.

Studies on the effects of GF on hepatic microsomal cytochrome P-450 and cytochrome b_5 in mice and rats have been reported [5, 6]. However, more extensive studies have been done on mice [7–9]. Studies in mice by Denk *et al.* [5] showed that GF feeding results in a significant increase in hepatic cytochrome b_5 and a decrease in cytochrome P-450. Studies by Lin *et al.* [6] showed that GF treatment results in an increase in benzo[*a*]pyrene hydroxylase activity and benzphetamine demethylation in mice.

Since drugs and xenobiotics have been shown to exhibit different effects on drug-metabolizing enzymes from different animal species, studies reported in this communication were done to more fully characterize the effects of GF on both Phase I and II drug-metabolizing enzymes in rats.

Materials and methods

Animals and treatment. Male Sprague–Dawley rats weighing 120–140 g were obtained from the Holtzman Co. (Madison, WI). All animals were kept under controlled conditions (22°, lights on 6:00 a.m. to 8:00 p.m.). Animals were fed a standard powdered Purina Chow diet containing 2.5% GF for 12 days. Control animals received powdered standard diet without GF. Feeding cups were weighed daily to monitor food intake by animals in both groups. Food intake was about equal in GF and control animals.

Chemicals. NADH, NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, cytochrome *c*, ferricyanide and griseofulvin were obtained from the Sigma Chemical Co. (St. Louis, MO). Benzphetamine was a gift from The Upjohn Co. (Kalamazoo, MI). All other reagents and chemicals employed were of analytical grade.

Enzyme assays. Liver microsomes were prepared following the procedure of Williams and Pendleton [10]. Total cytochrome P-450 was assayed according to the procedure of Omura and Sato [11]. Total cytochrome b_5 concentration was determined by addition of a few crystals of dithionite to the sample curvette [12]. Total heme was determined as pyridine hemochromogen according to the procedure of Paul *et al.* [13]. The metyrapone-difference spectrum of dithionite-reduced microsomes was recorded at room temperature on a Perkin–Elmer double beam, dual wavelength, scanning spectrophotometer according to the procedure of Luu-The *et al.* [14]. The assay procedures for NADPH–cytochrome *c*, NADH–cytochrome *c* and NADH–ferricyanide reductase activities of microsomes were done as previously described [15, 16]. Cytosolic glutathione *S*-transferase activity was assayed according to the procedure of Habig *et al.* [17]. Determination of benz-

phetamine demethylase activity was done following the procedures of Schenkman *et al.* [18]. Hepatic aryl hydrocarbon hydroxylase (AHH) activity was determined according to the procedure of Cantfort *et al.* [19] using ^{3}H-labeled benzo[*a*]pyrene, obtained from New England Nuclear (Boston, MA) as substrate. This substrate was greater than 98% pure. All spectrophotometric assays were done using a Perkin–Elmer double beam, dual wavelength scanning spectrophotometer, model 557. Protein was determined by the method of Lowry *et al.* [20].

Student's *t*-test was employed for all statistical analysis. Values are expressed as the mean ± S.D. P values greater than 0.05 were not considered statistically significant.

Results

Effects of GF on microsomal enzymes. Data in Table 1 show the effects of a 2.5% GF diet on various components of the hepatic microsomal electron transport system. Cytochrome P-450 levels were decreased significantly in microsomes from GF-treated rats compared to controls. GF feeding resulted in an approximate 2-fold increase in NADPH–cytochrome *c* reductase activity, whereas NADH–cytochrome *c* reductase activity was decreased by 50%. These results are partially consistent with those reported in studies on the mouse with respect to total heme, cytochrome P-450 concentration, and NADPH–cytochrome *c* reductase activity. However, the effect of GF on NADH–cytochrome *c* reductase activity in the rat is opposite that in the mouse.

Effects of GF on xenobiotic and drug metabolism. Data in Table 2 show the effects of dietary GF on hepatic aryl hydrocarbon hydroxylase (AHH) activity, an enzymic activity associated with Phase I drug metabolism. GF treatment resulted in an approximate 56% decrease in AHH activity compared to control. The amounts of microsomal protein per gram of liver were similar in both control and GF-treated animals. When NADPH was used as the only source of reducing equivalents, no significant difference in benzphetamine demethylase activity was seen in microsomes from control and GF-treated rats (Table 2). However, when NADPH and NADH were used as sources of reducing equivalents, benzphetamine demethylase activity in control microsomes was increased markedly. There was no significant change in the activity in microsomes from GF-treated rats. Data in Table 2 also show the effects of dietary GF on glutathionine *S*-transferase activity. This enzymic activity has been associated with Phase II of drug metabolism. Transferase activity was stimulated 2-fold in cytosol from GF-treated rats compared to controls.

Studies by Luu-The *et al.* [14] reported that metyrapone forms a spectrally apparent complex (λ_{max} = 446 nm) with dithionite-reduced cytochrome P-450$_b$ (the major phenobarbital-inducible hemoprotein) but not with other P-450 isoenzymes. We wished to ascertain the effects of dietary administration of GF on metyrapone binding to cytochrome P-450. Data in Table 3 show that GF treatment resulted in a significant increase in the metyrapone complex compared to control. Metyrapone bound to 86% of total cytochrome P-450 in the microsomes from GF-treated rats compared to 32% in control. To further compare the effects of GF, the metyrapone-binding affinity (K_s) of ferrocytochrome P-450 in microsomes from control and GF-treated rats was measured. The concentrations of metyrapone effecting half-maximum complex formation (K_s) were 10 and 3.5 μM for microsomes from control and GF-treated rats respectively (data not presented).

Discussion

Results of studies presented in this communication show that dietary administration of GF markedly altered the activities of enzymes associated with Phase I and II of drug metabolism (Tables 1 and 2). GF treatment has been shown to result in a decrease in cytochrome P-450 concentration and an increase in NADPH and NADH–cytochrome *c* reductase activity in mouse liver microsomes [5]. AHH activity is increased in these animals [6]. Results of our studies clearly show that GF treatment decreased both NADH–cytochrome *c* reductase and AHH activities in rat liver microsomes. Since neither NADH–ferricyanide reductase nor cytochrome b_5 concentration was affected by the GF treatment, the reason for the decrease in NADH–cytochrome *c* reductase is not clear. We have examined NADH-dependent stearyl CoA desaturase activity and NADH reduction of cytochrome P-450 in microsomes from

Table 1. Effects of griseofulvin (GF) diet on microsomal enzymes and cytochromes

Treatment	Total heme (nmoles/mg protein)	NADH–Ferricyanide reductase (μmoles/min/mg protein)	Cytochrome *c* reductase (nmoles/min/mg protein)		Cytochromes (nmoles/mg protein)	
			NADPH	NADH	b_5	P-450
Control	2.18 ± 0.15	4.98 ± 0.59	219 ± 39	1414 ± 179	0.46 ± 0.06	0.97 ± 0.14
GF	0.93 ± 0.13*	4.54 ± 0.51	409 ± 39†	705 ± 171†	0.48 ± 0.07	0.59 ± 0.07†

Animals were fed a diet containing 2.5% GF for 12 days. Control animals received a normal powdered rat chow diet. Values represent the mean ± S.D. of four or more separate experiments using two animals in each group. A total of eight to ten animals were used.

* Significantly different from control, $P < 0.001$.

† Significantly different from control, $P < 0.01$.

Table 2. Effects of griseofulvin (GF) diet on enzymic activities associated with Phase I and II of drug metabolism

Treatment	Aryl hydrocarbon hydroxylase activity (nmoles/min/mg protein)	Benzphetamine demethylase activity (nmoles HCHO/min/mg protein)		Glutathione *S*-transferase activity (nmoles/min/mg protein)
		NADPH	NADPH + NADH	
Control	0.99 ± 0.05	5.0 ± 0.4	7.0 ± 0.6	1049 ± 241
GF	0.43 ± 0.11*	4.5 ± 0.88	3.9 ± 0.5*	2217 ± 204*

Values represent the mean ± S.D. of four or more separate experiments using a total of eight to ten animals.

* Significantly different from control, $P < 0.01$.

Table 3. Effects of griseofulvin (GF) on the binding of metyrapone to dithionite-reduced microsomes

Treatment	Concentration of metyrapone complex* (μM)	Concentration of CO complex† (μM)	% Cytochrome P-450 bound to metyrapone
Control	0.61 ± 0.09	1.88 ± 0.05	32 ± 5
GF	0.85 ± 0.05‡	0.98 ± 0.09‡	86 ± 5‡

Animals were fed a diet containing 2.5% GF for 12 days. Control animals received normal powdered diet. Values represent the mean ± S.D. of five determinations.

* Calculated from the increase in absorbance at 446 nm – A_{490} using the millimolar extinction coefficient 52 $mM^{-1} \cdot cm^{-1}$ [14].

† Calculated from the increase in absorbance at 450 nm – A_{490} using the millimolar extinction coefficient 91 $mM^{-1} \cdot cm^{-1}$ [11].

‡ Significantly different from control, $P < 0.05$.

GF-treated rats (manuscript in preparation) and found these activities to be markedly different from control activities. Thus, the decreased NADH–cytochrome *c* reductase activity in microsomes from GF-treated rats may reflect an alteration in the flow of reducing equivalents from NADH. Further studies are required to fully explain this observation.

The decrease in total microsomal heme content and lack of a compensatory increase in cytochrome b_5 with a decrease in total microsomal cytochrome P-450 would suggest that GF treatment alters heme metabolism in rats. Conceivably the decrease in AHH activity might be due to a decrease in cytochrome P-448, the P-450 isozyme associated with AHH activity. The apparent decrease in benzphetamine demethylase activity appears to be due to a lack of NADH synergism in microsomes from GF-treated rats (Table 2). Results of our studies show that GF feeding markedly altered the binding of metyrapone to cytochrome P-450 (Table 3). Although total cytochrome P-450 was decreased approximately 50%, this decrease was not sufficient to account for the overall increase in the percent of cytochrome P-450 bound to metyrapone.

In conclusion, results of our studies suggest that dietary administration of GF markedly and differentially affects the activities of enzymes associated with Phase I and II of drug metabolism. Moreover, species differences exist in GF stimulation and inhibition of some of these enzymic activities between mice and rats.

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