ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1991, p. 1186–1190
0066-4804/91/061186-05$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 35, No. 6

Comparison of the Effects of the New Azalide Antibiotic, Azithromycin, and Erythromycin Estolate on Rat Liver Cytochrome P-450

DAVID E. AMACHER,[1]* SHELLI J. SCHOMAKER,[1] AND JAMES A. RETSEMA[2]

Drug Safety Evaluation Department[1] and Immunology and Infectious Diseases Department,[2] Pfizer Central Research, Groton, Connecticut 06340

Received 24 September 1990/Accepted 11 February 1991

Erythromycin and some other macrolide antibiotics can first induce a cytochrome P-450 isozyme similar to the one induced in rats by pregnenolone-16α-carbonitrile and then inhibit it by forming a stable cytochrome P-450–metabolite complex. The purpose of this study was to compare azithromycin, a novel 15-membered ring azalide, and erythromycin estolate for the potential to cause hepatic microsomal enzyme induction and inhibition in Sprague-Dawley rats. The daily oral administration of 800 mg of erythromycin estolate per kg for 7 days resulted in statistically significant elevations of NADPH-cytochrome *c* reductase, erythromycin *N*-demethylase (3.2-fold), and total cytochrome P-450 content. Approximately 40% of cytochrome P-450 was complexed with erythromycin metabolite. In contrast, the daily administration of 200 mg of azithromycin per kg for 7 days caused significant elevations of *N*-demethylase (2.5-fold) only and did not produce any increases in total cytochrome P-450 content or NADPH-cytochrome *c* reductase. No complexed cytochrome P-450 was detected in the azithromycin-dosed rats despite liver concentrations of azithromycin that were 118 times greater than the liver concentrations of erythromycin estolate in erythromycin estolate-dosed rats. Although the short-term oral administration of azithromycin produced hepatic accumulation of the drug and elevated azithromycin demethylase activity, there was no other evidence of hepatic cytochrome P-450 induction or inactivation via cytochrome-metabolite complex formation. In contrast to erythromycin estolate, azithromycin is not expected to inhibit its own metabolism or that of other drugs via this pathway.

Azithromycin is a new 15-membered ring azalide antibiotic (4) that differs from erythromycin by a methyl-substituted nitrogen at position 9a within the macrocyclic ring (19). This modification has resulted in improved stability at a low pH (9), improved potency against gram-negative organisms (19), and excellent in vivo activity against localized infections because of the high sustained tissue levels produced by azithromycin (10, 11).

A common characteristic of 14-membered ring macrolides such as erythromycin is the induction in rats of a hepatic cytochrome P-450 that is similar to the isozyme induced by certain glucocorticoids or antiglucocorticoids, such as dexamethasone or pregnenolone-16α-carbonitrile (PCN), respectively (6–14). For troleandomycin and erythromycin, the initial induction of microsomal cytochrome P-450 is accompanied by a substantial inhibition due to heme-macrolide metabolite complex formation (5, 17, 18). Sixteen-membered ring macrolides such as josamycin and midecamycin, on the other hand, do not significantly induce microsomal enzymes or form cytochrome P-450–metabolite complexes in rats (16).

The present study was done to determine whether azithromycin could induce or inhibit cytochrome P-450 in rat liver. The liver is of particular interest because of the very high tissue levels that can be obtained with this antibiotic after single or multiple doses (10, 20). For comparison, erythromycin estolate was also tested under conditions resulting in both the induction and the subsequent inhibition of microsomal cytochrome P-450.

* Corresponding author.

MATERIALS AND METHODS

Test compounds and dosing procedures. Six male CD [Crl:COBS CD (SD) BR] rats were dosed daily by oral gavage with 200 mg of azithromycin per kg for 7 days. This dose represents 10 times the amount administered to rats for 7 days in previous pharmacokinetic studies of this drug (20). Another six male CD rats were dosed with an equivalent volume (approximately 1 ml) of the vehicle (0.5% methylcellulose in water). Initially these animals weighed 160 to 170 g.

Six male CD rats were dosed daily by oral gavage with 800 mg of erythromycin estolate (Sigma E 8630) per kg for 7 days. This dose had previously been demonstrated to cause both cytochrome P-450 induction and cytochrome P-450 inhibition in rats after 7 days of dosing (21). Another six male CD rats were dosed with an equivalent volume (approximately 1 ml) of the vehicle (0.5% methylcellulose in water). Initially these animals weighed 160 to 170 g.

Three male CD rats were dosed daily with 75 mg of PCN per kg for 5 days. Microsomes from these rats were used for in vitro cytochrome P-450 binding studies with the macrolides. Another three male CD rats were dosed daily by oral gavage with 50 mg of PCN in 2% Tween 80–water per kg for 5 days (7). Three control animals received an equivalent volume of the vehicle for 5 days. Microsomes from these animals were used to test the effects of PCN on drug-metabolizing enzymes.

Preparation of microsomes. Animals were fasted overnight and then euthanized by sodium pentobarbital injection 24 h after the last treatment. Livers were perfused in situ with ice-cold phosphate-buffered saline (pH 7.4), removed, and weighed. Approximately 1-g quantities were set aside (at −80°C) for bioassay. Liver concentrations of azithromycin and erythromycin were determined by an agar well diffusion

bioassay with *Micrococcus luteus* ATCC 9341 as the bioassay organism (10). The sensitivity of the assay for both azithromycin and erythromycin was approximately 0.1 μg/ml. Samples from azithromycin-treated rats were diluted in liver homogenate from control rats to obtain zone sizes that were in the linear section of the bioassay curve.

Excised livers were minced in 0.25 M sucrose–0.05 M Tris (pH 7.4) and homogenized. Individual livers were processed for microsome preparation by the procedure of Elshourbagy and Guzelian (7). Microsomes were removed from the ultracentrifuge, quickly frozen in a dry ice-isopropanol bath, and stored at −80°C.

Analytical procedures. Microsomal protein content was determined by the bicinchoninic acid method (standard protocol) modified for a Cobas-bio autoanalyzer and using Pierce reagents. The applicable protein concentration range for this assay is 20 to 1,200 μg/ml. Protein standards of known concentrations were prepared by diluting a stock solution of bovine serum albumin. To each 10 μl of standard or unknown protein sample, 200 μl of Pierce working reagent (an equal mixture of two preformulated solutions containing sodium carbonate, sodium bicarbonate, bicinchoninic acid detection reagent, sodium tartrate, NaOH, and copper sulfate) was added. The samples were mixed well and incubated at 37°C, and the A_{562} was read. Independent protein calibration standards run with each assay indicated instrumental accuracies of 108% ± 3%, 98% ± 1%, and 95% ± 1% for 250, 500, and 750 μg of protein per ml, respectively (n = 13).

Hepatic microsomes were assayed for noncomplexed cytochrome P-450 (difference between A_{450} and A_{490} per milligram of protein) by the CO-reduced difference spectrum procedure of Omura and Sato (15) with a Beckman DU7 spectrophotometer and the modified procedure of Guengerich (12). Total cytochrome P-450 (complexed plus noncomplexed) was also determined by the Omura-Sato procedure after the addition of 50 μM potassium ferricyanide to both reference and sample cuvettes (13). The latter procedure is used to release any cytochrome that is complexed with macrolide metabolites (21). Complexed cytochrome P-450 (difference between A_{456} and A_{490} per milligram of protein) was measured after the addition of 50 μM potassium ferricyanide to the reference cuvette (3). Under the latter conditions, the presence of a Soret peak at 456 nm indicates the formation of a stable P-450–Fe(II)–metabolite complex (6). Results were expressed as nanomoles of cytochrome P-450 per milligram of protein.

The binding of azithromycin or erythromycin estolate to rat liver microsomes (2 mg of microsomal protein per ml in 0.1 M phosphate buffer [pH 7.4]) prepared from untreated and PCN-dosed animals was analyzed by differential spectroscopy after the addition of NADPH. Previous determinations of the cytochrome content from these PCN-dosed animals indicated an elevated cytochrome P-450 content, but no complexed cytochrome P-450 was present. Timed 380- to 500-nm scans (5 min each) in the presence or absence of 0.6 mM erythromycin estolate or azithromycin were recorded.

NADPH-cytochrome c reductase was measured in the Cobas-bio autoanalyzer by an adaptation of the procedure of Guengerich (12). Microsome samples were resuspended in 0.1 M potassium phosphate buffer (pH 7.4). The 0.5 mM horse heart cytochrome c (Sigma) reagent was prepared in 10 mM potassium phosphate buffer (pH 7.7). Enzyme samples and the cytochrome c reagent were mixed and preincubated at 30°C for 2 min prior to the addition of NADPH. After the addition of NADPH, the A_{550} was monitored every 10 s for a total of 10 determinations. The linear correlation over this

TABLE 1. Effects of erythromycin estolate on hepatic drug-metabolizing enzymes

Treatment group (no. of animals)	Erythromycin in liver (μg/g)	Liver wt/body wt (%)	NADPH-cytochrome c reductase (nmol/min/mg of protein)	Cytochrome P-450 (nmol/mg of protein)			*N*-Demethylase activity with the following substrate:		HCHO (nmol/mg in 15 min) formed from erythromycin[a]	
				Noncomplexed	Complexed	Total[b]	Azithromycin	Erythromycin	Without $K_3Fe(CN)_6$	With $K_3Fe(CN)_6$
Control (6)[c]		3.3 ± 0.3	243 ± 31	0.752 ± 0.369	0 ± 0	0.676 ± 0.128	8.35 ± 3.91	7.23 ± 4.73		2.39 ± 0.99
Erythromycin estolate (800 mg/kg daily for 7 days) (6)[c]	13.0 ± 4.3	3.5 ± 0.2	355 ± 49	0.815 ± 0.138	0.665 ± 0.197	1.710 ± 0.398	21.60 ± 4.72	23.43 ± 4.43	14.80 ± 3.32	28.68 ± 12.07
Two-sample t-test P value		0.106	0.0085	0.7006		0.00012	0.004	0.0001		0.0082

[a] Microsomes had been frozen for 43 days.
[b] Noncomplexed, complexed, and total cytochrome P-450 contents were determined independently for each sample. For this reason, noncomplexed plus complexed does not equal total.
[c] Values are reported as means ± standard deviations.

range was 0.996. Results were expressed as nanomoles of product formed per milligram of protein per minute.

N-Demethylase activity with either erythromycin or azithromycin as the substrate was determined by the procedure of Watkins et al. (22). The initial reaction mixture contained 1 mg of microsomal protein per ml in 0.1 M potassium phosphate buffer (pH 7.4) and either 0.4 mM erythromycin or 0.4 mM azithromycin as the substrate. The reaction was started by the addition of 1 mM NADPH (final concentration) and stopped by the addition of 0.4 ml of 17% $HClO_4$. The supernatant was heated with Nash reagent (12) and cooled, and the A_{412} was read and compared with those of formaldehyde standards. Results were expressed as nanomoles of formaldehyde formed per milligram of microsomal protein in 15 min.

RESULTS

For the erythromycin estolate-dosed animals, the mean daily weight gain was 0 g; that of concurrently tested control rats was 8 g. No other assessment of general toxicity was made in this study, and food consumption was not measured. For the azithromycin-dosed animals, both control and treated rats had a mean daily weight gain of 10 g.

The effects of erythromycin estolate upon the hepatic drug-metabolizing enzymes of the rats are summarized in Table 1. Significant differences ($P < 0.05$) compared with controls were found in the NADPH-cytochrome *c* reductase activities, the total cytochrome P-450 content [in the presence of $K_3Fe(CN)_6$], and the *N*-demethylase activities with either substrate. *N*-Demethylase activities were significantly greater in the presence of potassium ferricyanide. In general, erythromycin *N*-demethylase activities were rather low in this study, with some control values at or near the limits of detection by the colorimetric procedure of Watkins et al. (22). Overall, the control *N*-demethylase activities with erythromycin as a substrate ranged from 2 to 21 nmol of HCHO per mg in 15 min. The liver weights of the erythromycin estolate-dosed animals did not differ from those of the control animals.

The effects of azithromycin upon the hepatic drug-metabolizing enzymes of the rats are summarized in Table 2. Significant differences ($P < 0.05$) compared with controls were found in the *N*-demethylase activities with either substrate. No complexed cytochrome P-450 was detected in control or azithromycin-dosed animals, and total cytochrome P-450 content and NADPH-cytochrome *c* reductase activities in the treated animals did not differ from those in the control animals. Also, the liver weights of the treated animals did not differ from those of the control animals. The mean concentration of azithromycin in the liver was much higher (1,531 μg/g) than the mean concentration of erythromycin in the liver (13 μg/g).

In contrast to azithromycin, PCN produced significant increases in relative liver weights and total cytochrome P-450 content but did not elevate erythromycin *N*-demethylase activity (Table 3). Azithromycin *N*-demethylase activity was not assayed. Binding experiments with either erythromycin or azithromycin and microsomes from PCN-dosed rats showed absorption minima at 411 nm and absorption maxima at 425 nm. Only erythromycin caused small increases in the A_{425} over time (data not shown), suggesting binding.

TABLE 2. Effects of azithromycin on hepatic drug-metabolizing enzymes

Treatment group (no. of animals)	Azithromycin in liver (μg/g)	Liver wt/body wt (%)	NADPH-cytochrome *c* reductase (nmol/min/mg of protein)	Cytochrome P-450 (nmol/mg of protein)			*N*-Demethylase activity with the following substrate:		HCHO (nmol/mg in 15 min) formed from azithromycin[a]	
				Noncomplexed	Complexed	Total[b]	Azithromycin	Erythromycin	Without $K_3Fe(CN)_6$	With $K_3Fe(CN)_6$
Control (6)[c]		3.4 ± 0.2	296 ± 25	0.749 ± 0.286	0 ± 0.124	0.600	5.18 ± 1.55	7.90 ± 5.30	14.19 ± 3.91	6.61 ± 2.08
Azithromycin (200 mg/kg daily for 7 days) (6)[c]	1,531 ± 207	3.3 ± 0.1	323 ± 52	0.590 ± 0.213	0	0.631 ± 0.177	12.56 ± 4.60	17.20 ± 5.21	22.62 ± 7.33	16.31 ± 1.17
Two-sample *t*-test *P* value		0.093	0.269	0.301		0.718	0.0098	0.0118	0.0322	0.0001

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *b*.
[c] See Table 1, footnote *c*.

TABLE 3. Effects of PCN on hepatic drug-metabolizing enzymes

Treatment group (no. of animals)	Liver wt/body wt (%)	NADPH-cytochrome *c* reductase (nmol/min/mg)	Cytochrome P-450 (nmol/mg)	Erythromycin *N*-demethylase activity (nmol of HCHO per mg in 15 min)
Control (3)[a]	3.3 ± 0.4	312 ± 22	1.05 ± 0.06	21 ± 1.4
PCN (50 mg/kg daily for 5 days) (3)[a]	3.9 ± 0.2	354 ± 49	1.92 ± 0.31	24 ± 2
Two-sample *t*-test *P* value	0.04	0.246	0.0087	0.147

[a] Values are reported as means ± standard deviations.

DISCUSSION

Erythromycin, troleandomycin, and other macrolactones with a tertiary amine function can affect hepatic cytochrome P-450 in two ways: (i) their administration to humans or rats can lead to the induction of a specific cytochrome P-450 isozyme that is also specifically induced in rats after the administration of dexamethasone or the antiglucocorticoid PCN, and (ii) they can selectively inhibit this cytochrome P-450 form after oxidation of their dimethylamino group into a nitrosoalkane metabolite by the formation of a stable cytochrome P-450–Fe(II)–metabolite complex (6, 14). In rats, this specific isozyme is designated cytochrome P-450p, is associated with testosterone-6β-hydroxylase, UDP-glucuronosyltransferase, and erythromycin *N*-demethylase activities, and is homologous to cytochrome HLp in humans (1, 8). Sixteen-membered ring macrolides such as josamycin and midecamycin do not significantly induce microsomal enzymes and do not form cytochrome P-450–metabolite complexes in rats (16). The ansamycin rifampin can induce cytochrome P-450p but cannot form inhibitory cytochrome heme-metabolite complexes (14). The consequence of the dual induction and inhibition phenomenon is that it could lead to decreased elimination of the drug and, hence, secondary toxic effects or to macrolide interactions with other cytochrome P-450p substrates, such as carbamazepine, ergotamine, theophylline, and perhaps cyclosporin A (2, 14, 16).

In this study, the daily oral administration of 800 mg of erythromycin estolate per kg resulted in the significant elevation of NADPH-cytochrome *c* reductase, *N*-demethylase, and total cytochrome P-450 when measured in the presence of potassium ferricyanide. A substantial portion of this induced cytochrome P-450 was complexed, presumably with the macrolide metabolite. This treatment with erythromycin estolate had no effect on the relative liver weight but caused a marked inhibition of daily weight gain during the 7 days of exposure. In contrast, the daily oral administration of 200 mg of azithromycin per kg had no measurable effect on total cytochrome P-450 or NADPH-cytochrome *c* reductase, and only small but significant elevations in *N*-demethylase were noted. These results were also in contrast to the effects of PCN on liver weight, cytochrome P-450, and *N*-demethylase activity.

Although levels in serum were not measured in this study, azithromycin has been shown to produce higher peak levels in serum and much higher values for areas under the curve in rat serum than do equal doses of erythromycin (10, 20). In comparison with erythromycin, azithromycin produces high sustained tissue levels leading to values for areas under the curve in rat liver that are 65 times higher than those of erythromycin (10). In this study, liver exposure to azithromycin was much higher than that to erythromycin, as measured by concentrations in liver tissue; azithromycin levels were 118 times higher 24 h after the last azithromycin dose than erythromycin levels in the livers of erythromycin estolate-dosed animals (Table 2). Spectral analysis of suspensions of microsomes from PCN-dosed animals in the presence of equimolar concentrations of either macrolide indicated some binding of erythromycin but not azithromycin, as suggested by small increases in the A_{425} over time. Thus, unlike PCN or erythromycin estolate, azithromycin did not induce, inhibit, or bind to cytochrome P-450, even though substantial concentrations of this azalide were noted in the liver.

In summary, these results indicate that the short-term (7-day) oral administration of nontoxic doses of azithromycin produces substantial hepatic concentrations of the drug and significant elevations of *N*-demethylase activity but, unlike erythromycin, produces no other evidence of hepatic cytochrome P-450 induction or inactivation via cytochrome-metabolite complex formation. In contrast to erythromycin, azithromycin is not expected to inhibit its own metabolism or that of other drugs via hepatic cytochrome P-450 induction.

REFERENCES

1. **Arlotto, M. P., A. J. Sonderfan, C. D. Klaassen, and A. Parkinson.** 1987. Studies on the pregnenolone-16α-carbonitrile-inducible form of rat liver microsomal cytochrome P-450 and UDP-glucuronosyltransferase. Biochem. Pharmacol. **36**:3859–3866.
2. **Bertault-Peres, P., C. Bonfils, G. Fabre, S. Just, J. Cano, and P. L. Maurel.** 1987. Metabolism of cyclosporine A. II. Implication of the macrolide antibiotic inducible cytochrome P-450 3c from rabbit liver microsomes. Drug Metab. Dispos. **15**:391–398.
3. **Beuning, M. K., and M. R. Franklin.** 1976. SKF 525-A inhibition, induction and 452-nm complex formation. Drug Metab. Dispos. **4**:244–255.
4. **Bright, G. M., A. A. Nagel, J. Bordner, K. A. Desai, J. N. Dibrino, J. Nowakowska, L. Vincent, R. M. Watrous, F. C. Sciavolino, A. R. English, J. A. Retsema, M. R. Anderson, L. A. Brennan, R. J. Borovoy, C. R. Cimochowski, J. A. Faiella, A. E. Girard, D. Girard, C. Herbert, M. Manousos, and R. Mason.** 1988. Synthesis, *in vitro* and *in vivo* activity of novel 9-deoxo-9a-aza-9a-homoerythromycin A derivatives: a new class of macrolide antibiotics, the azalides. J. Antibiot. **41**:1029–1047.
5. **Danan, G., V. Descatoire, and D. Pessayre.** 1981. Self-induction by erythromycin of its own transformation into a metabolite forming an inactive complex with reduced cytochrome P-450. J. Pharmacol. Exp. Ther. **218**:509–514.
6. **Delaforge, M., E. Sartori, and D. Mansuy.** 1988. *In vivo* and *in vitro* effects of a new macrolide antibiotic roxithromycin on rat liver cytochrome P-450: comparison with troleandomycin and erythromycin. Chem. Biol. Interact. **68**:179–188.
7. **Elshourbagy, N. A., and P. S. Guzelian.** 1980. Separation, purification and characterization of a novel form of hepatic cytochrome P-450 from rats treated with pregnenolone-16α-carbonitrile. J. Biol. Chem. **255**:1279–1285.
8. **Fabre, G., P. Crevat-Pisano, S. Dragna, J. Covo, Y. Barra, and J. P. Cano.** 1988. Involvement of the macrolide antibiotic inducible cytochrome P-450 LM_{3c} in the metabolism of midazolam by microsomal fractions prepared from rabbit liver. Bio-

chem. Pharmacol. **37**:1947–1953.
9. **Fiese, E. F., and S. H. Steffen.** 1990. Comparison of the acid stability of azithromycin and erythromycin. A. J. Antimicrob. Chemother. **25**(Suppl. A):39–47.
10. **Girard, A. E., D. Girard, A. R. English, T. D. Gootz, C. R. Cimochowski, J. A. Faiella, S. L. Haskell, and J. A. Retsema.** 1987. Pharmacokinetic and in vivo studies with azithromycin (CP-62,993), a new macrolide with an extended half-life and excellent tissue distribution. Antimicrob. Agents Chemother. **31**:1948–1954.
11. **Girard, A. E., D. Girard, and J. A. Retsema.** 1990. Correlation of the extravascular pharmacokinetics of azithromycin with in-vivo efficacy in models of localized infection. J. Antimicrob. Chemother. **25**(Suppl. A):61–71.
12. **Guengerich, F. P.** 1982. Microsomal enzymes involved in toxicology—analysis and separation, p. 609–634. *In* A. W. Hayes (ed.), Principles and methods of toxicology. Raven Press, New York.
13. **Larrey, D., M. Tinel, and D. Pessayre.** 1983. Formation of inactive cytochrome P-450 Fe(II)-metabolite complexes with several erythromycin derivatives but not with josamycin and midecamycin in rats. Biochem. Pharmacol. **32**:1487–1493.
14. **Mansuy, D.** 1987. Formation of reactive intermediates and metabolites: effects of macrolide antibiotics on cytochrome P-450. Pharmacol. Ther. **33**:41–45.
15. **Omura, T., and R. Sato.** 1964. The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. Biol. Chem. **239**:2370–2378.
16. **Pessayre, D.** 1983. Effects of macrolide antibiotics on drug metabolism in rats and in humans. Int. J. Clin. Pharmacol. Res. **3**:449–458.
17. **Pessayre, D., V. Descatoire, M. Konstantinova-Mitchevam, J. C. Wandscherr, B. Cobert, R. Level, J. P. Benhamou, M. Jaouen, and D. Mansuy.** 1981. Self-induction by triacetyloleandomycin of its own transformation into a metabolite forming a stable 456 nm-absorbing complex with cytochrome P-450. Biochem. Pharmacol. **30**:553–558.
18. **Pessayre, D., M. Konstantinova-Mitchevam, V. Descatoire, C. Cobert, J. C. Wandscherr, R. Level, G. Feldman, D. Mansuy, and J. P. Benhamou.** 1981. Hypoactivity of cytochrome P-450 after triacetyloleandomycin administration. Biochem. Pharmacol. **30**:559–564.
19. **Retsema, J., A. Girard, W. Schelkly, M. Manousos, M. Anderson, G. Bright, R. Borovoy, and R. Mason.** 1987. Spectrum and mode of action of azithromycin (CP-62,993), a new 15 membered-ring macrolide with improved potency against gram-negative organisms. Antimicrob. Agents Chemother. **31**:1939–1947.
20. **Shepard, R. M., and F. C. Falkner.** 1990. Pharmacokinetics of azithromycin in rats and dogs. J. Antimicrob. Chemother. **25**(Suppl. A):49–60.
21. **Villa, P., D. Sassella, M. Corada, and I. Bartosek.** 1988. Effects of roxithromycin, a new semisynthetic macrolide, and two erythromycins on drug metabolizing enzymes in rat liver. J. Antibiot. **41**:563–569.
22. **Watkins, P. B., S. A. Wrighton, P. Maurel, E. G. Schuetz, G. Mendez-Picon, G. A. Parker, and P. S. Guzelian.** 1985. Identification of an inducible form of cytochrome P-450 in human liver. Proc. Natl. Acad. Sci. USA **82**:6310–6314.