Cytochrome P450 3A4 and P-glycoprotein mediate the interaction between an oral erythromycin breath test and rifampin

Background: The intravenous ^{14}C-erythromycin breath test ($ERMBT_{IV}$) does not measure aggregate liver and intestinal cytochrome P450 (CYP) 3A4 activity. Accordingly, we evaluated an oral stable-labeled (^{13}C) formulation of the test ($ERMBT_{oral}$) as an alternative CYP3A4 phenotyping probe.

Methods: After an overnight fast, 14 young healthy volunteers (5 women and 9 men) received the $ERMBT_{IV}$ (0.07 μmol, 3 μCi), followed by the $ERMBT_{oral}$ (500 mg). The next morning, the CYP3A4 inhibitor troleandomycin (500 mg) was given, and both ERMBTs were repeated. After at least 24 hours, the CYP3A4 and P-glycoprotein inducer rifampin (600 mg; INN, rifampicin) was given daily for 7 days, and both ERMBTs were repeated 24 hours after the last dose of rifampin. Plasma samples were collected for 10 hours with each administration of the $ERMBT_{oral}$, and erythromycin levels were measured by liquid chromatography–mass spectrometry. Finally, the effect of troleandomycin on erythromycin transport was examined in Caco-2 cell monolayers.

Results: Compared with baseline values, the median $ERMBT_{IV}$ and $ERMBT_{oral}$ results and erythromycin apparent oral clearance (CL/F) all significantly decreased, by at least 70%, with troleandomycin treatment ($P = .001$ for each comparison). With rifampin treatment, the median $ERMBT_{IV}$ result and CL/F increased 2-fold ($P \leq .01$), but the median $ERMBT_{oral}$ result was unchanged ($P = .30$). There were no rank-order correlations between the $ERMBT_{IV}$ and $ERMBT_{oral}$ results or between either ERMBT result and CL/F within each treatment group ($P \geq .07$). In addition, troleandomycin had no effect on erythromycin transport in Caco-2 cells ($P \geq .20$).

Conclusions: The $ERMBT_{oral}$ was influenced by processes in addition to intestinal and hepatic CYP3A4 activity and therefore did not provide a straightforward measure of aggregate CYP3A4 phenotype. The erythromycin-rifampin interaction cannot be attributed to CYP3A4 induction alone and probably also reflected intestinal P-glycoprotein induction. (Clin Pharmacol Ther 2002;72:524-35.)

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The intravenous erythromycin breath test ($ERMBT_{IV}$) was developed more than a decade ago as a simple and relatively noninvasive index of cytochrome P450 (CYP) 3A4 activity in vivo.[1] The theory behind this test stems from the observation in human liver microsomes that CYP3A4 is the major enzyme involved in the primary metabolic pathway of erythromycin, *N*-demethylation.[1] Following this common CYP-mediated oxidative reaction, the cleaved methyl group, as formaldehyde, is further enzymatically converted to bicarbonate, which then equilibrates with alveolar carbon dioxide.[2] Because the $ERMBT_{IV}$ uses [^{14}C-*N*-methyl]erythromycin, $^{14}CO_2$ is liberated in breath. (A complete scheme of the various steps involved in the CYP3A4-mediated production of $^{14}CO_2$ from [^{14}C-*N*-methyl]erythromycin was recently described by Rivory et al.[3]) By calculation of the rate of appearance of $^{14}CO_2$ in breath, as early as 20 minutes after injection,[4] an indirect but rapid measure of CYP3A4 activity is obtained.

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The $ERMBT_{IV}$ has been shown to predict a significant portion of the interindividual variation in the disposition of some CYP3A4 substrates, including the immunosuppressant cyclosporine (INN, ciclosporin),[5] the nonnucleoside reverse transcriptase inhibitor delavirdine[6] and, most recently, the chemotherapeutic agent docetaxel.[7] In addition, the $ERMBT_{IV}$ appears to be sensitive to the effects of CYP3A4 inducers, such as glucocorticoids and rifampin (INN, rifampicin),[1,8,9] and especially to a variety of CYP3A4 inhibitors. Ketoconazole, clarithromycin, amprenavir, delavirdine, and troleandomycin have all been shown to reduce average $ERMBT_{IV}$ results by at least 60% in comparison with baseline values.[1,6,10-12] These findings suggest that this test could be particularly useful in the prediction of drug-drug interactions in which CYP3A4 is a major determinant.

In addition to being highly expressed in the liver, CYP3A4 is highly expressed in the small intestine.[13] The $ERMBT_{IV}$ result would, therefore, appear to reflect only hepatic CYP3A4 activity because intravenous administration should avoid significant metabolism by the gut. Indeed, the average rate at which $^{14}CO_2$ was exhaled by patients with severe liver disease was just 16% of that in subjects with normal liver function,[14] and $^{14}CO_2$ production was negligible during the anhepatic phase of a liver transplant operation.[15] Therefore, if the goal were to assess aggregate intestinal and hepatic CYP3A4 activity, the $ERMBT_{IV}$ alone would not suffice. Accordingly, we developed an oral stable isotope (^{13}C)-labeled formulation of the ERMBT ($ERMBT_{oral}$). We began our validation of this test by examining the effects of a prototypical CYP3A4 inhibitor (troleandomycin) and inducer (rifampin) on the rate of $^{13}CO_2$ production. This investigation further revealed that mechanisms in addition to CYP3A4-mediated metabolism govern the erythromycin-rifampin interaction.

MATERIAL AND METHODS

Materials and chemicals

Uncoated track-etched polyethylene terephthalate inserts and murine laminin were purchased from Collaborative Biochemical Products (Bedford, Mass). Dulbecco's modified Eagle medium with high glucose, Hanks balanced salt solution, and nonessential amino acids were purchased from Invitrogen (Carlsbad, Calif). Fetal bovine serum was purchased from Hyclone (Logan, Utah). Gentamicin (in lieu of penicillin-streptomycin), dl-α-tocopherol, sodium selenite, zinc sulfate, unlabeled digoxin, unlabeled erythromycin, troleandomycin, and oleandomycin were purchased from Sigma Chemical Co (St Louis, Mo). [^{3}H]Digoxin (19 Ci/mmol) was purchased from New England Nuclear (Boston, Mass). [^{14}C-*N*-methyl]erythromycin (54.5 mCi/mmol) was purchased from Metabolic Solutions, Inc (Nashua, NH). [$^{13}C_2$-*N,N*-methyl]erythromycin was obtained from Cambridge Isotope Laboratories, Inc (Andover, Mass). Before use, the bulk material was analyzed by high-resolution mass spectrometry (to determine identity), combustion isotope ratio mass spectrometry (to determine ^{13}C enrichment), and HPLC (to determine purity); weighed in 500-mg quantities; and then placed into unit-dose capsules. LY335979 [full chemical name, (2*R*-anti-5-{3-[4-(10,11-difluoromethanodibenzo-suber-5-yl)piperazin-1-yl]-2-hydroxypropoxy} quinoline trihydrochloride)], a selective P-glycoprotein inhibitor,[16] was a gift from Eli Lilly and Co (Indianapolis, Ind). All other chemicals and reagents were of tissue culture or reagent grade when appropriate.

Reproducibility of the $ERMBT_{oral}$

To evaluate the reproducibility of the $ERMBT_{oral}$, a preliminary investigation was conducted in 15 healthy nonsmoking volunteers who were maintained on their usual diets. The test was administered to each subject on 3 separate occasions—days 1, 2, and 8. On the morning of each day, a single oral dose of [$^{13}C_2$-*N,N*-methyl]erythromycin (500 mg, equivalent to 1.14 mmol erythromycin base), was given with 3 Maalox (Novartis, East Hanover, NJ) tablets (to neutralize stomach acid and minimize erythromycin degradation) and 8 ounces of water. The subject was instructed to exhale through a Quintron disposable modified Haldane-Priestley tube (Milwaukee, Wis) that was connected to a plastic bag. Breath samples were collected into Exetainer breath storage tubes (Labco Ltd, High Wycombe, United Kingdom) just before dose administration and at 30-minute intervals for 3 hours thereafter. Each breath sample was analyzed for $^{13}CO_2$ content by mass spectrometry (see "Analytic procedures").

Human subject study

Subjects. A separate group of healthy nonsmoking volunteers (5 women and 9 men; age range, 21-43 years; weight range, 47-83 kg) participated in the study. Before enrollment, each subject had normal findings after a routine physical examination and laboratory tests that included hematologic testing, serum chemical

testing, and urinalysis. In addition, none of the women were of childbearing potential. No subject was taking other medications, either prescription or nonprescription, for at least 2 weeks before and during the study, and no subject had known allergies to macrolide antibiotics or rifampin. All subjects were instructed to refrain from consuming grapefruit-containing products and alcoholic beverages beginning at least 2 days before and during the course of the study. The University of Michigan Institutional Review Board approved the study, and all subjects provided written informed consent.

Study design. This study consisted of 3 phases, each preceded by an overnight fast. On the morning of the first phase (day 1), an indwelling heparin-lock catheter was placed into an arm vein of each subject for blood collection. The subject was then administered an intravenous dose of [^{14}C-*N*-methyl]erythromycin (0.07 μmol, 3 μCi) into the opposite arm. Subsequently, a single oral dose of [$^{13}C_2$-*N,N*-methyl]erythromycin was administered. Blood (5 mL) was collected just before oral drug administration and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 10 hours thereafter. Plasma was separated from blood cells by centrifugation and was stored at −20°C, pending analysis for erythromycin. The second phase began the following morning (day 2) when the subject was given a single oral dose (500 mg) of troleandomycin (TAO; Pfizer Inc, New York, NY). Two hours later and at the same times used for the previous day's tests, the $ERMBT_{IV}$ and $ERMBT_{oral}$ were administered. Blood sample collections were repeated. Phase 3 began the next morning (day 3) or up to 7 days later when the subject was given a single oral dose (600 mg) of rifampin (Rifadin; Aventis Pharmaceuticals, Kansas City, Mo). For the next 6 consecutive mornings, the subject was given the same oral dose of rifampin. On the day after the final rifampin dose, both ERMBTs and blood sample collections were repeated. During all 3 phases, the subject was not allowed to eat until after the 4-hour blood sample was drawn.

Intravenous and oral erythromycin breath tests. For the $ERMBT_{IV}$, before and 20 minutes after injection, each subject was instructed to exhale through a tube into a scintillation vial that contained a CO_2 trapping solution (hyamine and ethanol) as described previously.[1] Expired $^{14}CO_2$ was measured by liquid scintillation counting. Results are expressed as the percentage of administered ^{14}C exhaled during the first hour after injection, as estimated by a single breath collection at 20 minutes.[17] The $ERMBT_{oral}$ was administered and breath was collected during the ensuing 3 hours exactly as described for the reproducibility study. Results are expressed as the percentage of the dose exhaled as $^{13}CO_2$ during the interval from 0 to 3 hours.

Analytic procedures

$^{13}CO_2$ in breath after $ERMBT_{oral}$ administration. The amount of $^{13}CO_2$ in the breath storage tubes was determined by use of a Europa Scientific 20/20 gas isotope ratio mass spectrometer (Cincinnati, Ohio). The ratio of $^{13}CO_2$ to $^{12}CO_2$ (mass 45 and 44, respectively) was measured in each sample and compared with a reference gas (5% CO_2, balanced with 75% N_2 and 20% O_2). The reference gas was calibrated with international standards. The units of measurement were atom percentage of ^{13}C expired (APE) and were defined by the following equation:

$$APE = {}^{13}CO_2/({}^{13}CO_2 + {}^{12}CO_2) \times 100\%$$

Standards of carbon dioxide gas at 3 different levels of APE were analyzed before and after each daily run to assess instrument performance. The analytic precision of the instrument was 0.0001 APE. The APE values from each breath sample were used to calculate the percentage of the dose recovered during each time interval. The area under the APE-versus-time curve for each of the 0- to 1-, 1- to 2-, and 2- to 3-hour intervals (AUC_{breath}) was calculated by use of the linear trapezoidal method. The percentage of the dose metabolized during each 1-hour interval was calculated as the ratio of the total amount of ^{13}C recovered (in millimoles) in that interval ($^{13}C_{interval}$) to the administered dose (1.14 mmol), for which $^{13}C_{interval}$ was calculated according to the following equation:

$$\begin{aligned} {}^{13}C_{interval}\ (mmol) = {} & AUC_{breath}\ (h) \\ & \times CO_2\ \text{production rate}\ (mmol/min/m^2\ BSA) \\ & \times BSA\ (m^2) \times 60\ min/h \end{aligned}$$

in which BSA is body surface area. The CO_2 production rate was assumed to be 5 mmol CO_2/min/m^2 BSA.[18]

Erythromycin in plasma after oral administration. The concentration of erythromycin in plasma was determined by use of HPLC with mass spectrometric detection. Plasma (100 μL) was spiked with 300 ng oleandomycin as the internal standard. Samples were mixed by vortex for approximately 10 seconds and then centrifuged for 5 minutes at 14,000*g*. The supernatant was recovered, and a 5-μL aliquot was injected onto a Hewlett Packard 1090 Series II liquid chromatography system (Palo Alto, Calif) coupled to a Sciex API III mass spectrometer (Thornhill, Ontario, Canada). Analytes were separated with a Phenomenex IB-SIL C_{18}-BD column (3 μm, 3.3 cm × 4.6 mm; Torrance,

Calif). The mobile phase, consisting of methanol and 25-mmol/L ammonium acetate (76:24), was delivered at a constant flow rate of 1 mL/min. The effluent flowed directly into the electrospray ion source of the mass spectrometer, which was operated in the selected ion monitoring mode. The $[M+H]^+$ ions at mass-to-charge ratios of 736.7 ($^{13}C_2$-erythromycin) and 688.7 (oleandomycin) were monitored. A set of 8 nonzero calibration standards that ranged from 20 to 2000 ng/mL and quality control samples at concentrations of 60, 800, and 1700 ng/mL were run during each day of analysis. The calibration curve was linear over the range studied, with a correlation coefficient $\geq$0.995. Samples with nominal concentrations above the highest calibration standard were diluted and reanalyzed in a subsequent batch. The interassay coefficients of variation for the quality control samples were 10.9%, 3.8%, and 4.1% for the low-, middle-, and high-quality control samples, respectively. The accuracy of the assay ranged from 93% to 99% nominal of the known standard. The low volume injected onto the liquid chromatography–mass spectrometry system did not compromise the chromatography or the ionization of the analytes for the duration of analysis.

Pharmacokinetic analysis

The pharmacokinetics of erythromycin after oral administration were evaluated by use of noncompartmental methods. The terminal elimination rate constant (λ_Z) was determined by log-linear regression of at least the last 3 data points from the plasma concentration–time profile. The terminal elimination half-life ($t_{1/2}$) was calculated as $\ln 2/\lambda_Z$. The area under the plasma concentration–time curve (AUC) was calculated by use of the linear trapezoidal method and extrapolation to infinite time by dividing the last measured concentration by λ_Z. The apparent oral clearance (CL/F) was calculated as the ratio of dose to AUC. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were obtained by visual inspection of the concentration–time profile.

Evaluation of troleandomycin as an inhibitor of erythromycin transport

Cell culture inserts were coated with the extracellular matrix protein laminin (5 $\mu g/cm^2$), as described previously,[19] onto which the Caco-2 cell clone P27.7,[20] passage 33, was seeded at a density of approximately 5×10^5 cells/cm^2. When confluence was achieved (transepithelial electrical resistance values $\geq$250 $\Omega \cdot cm^2$), the cell monolayers were treated for 2 weeks with differentiation medium (devoid of 1α,25-dihydroxyvitamin D_3) as described previously.[19,20] Incubation medium (serum-free differentiation medium) was freshly prepared and kept at 37°C. LY335979, troleandomycin, and unlabeled digoxin were dissolved as 1000-fold concentrated solutions in dimethyl sulfoxide. [^{3}H]-Digoxin was further diluted in absolute ethanol. Unlabeled erythromycin was dissolved as a 100-fold concentrated solution in ethanol. The contents of 4 single-dose vials of [^{14}C-*N*-methyl]erythromycin were combined and concentrated (about 30-fold) under a gentle stream of nitrogen to deliver 0.5 μCi of radioactivity per culture insert while minimizing the final concentration of ethanol.

Incubation medium was spiked either with drug(s) or with vehicle (dimethyl sulfoxide or ethanol), or with both, just before its addition to triplicate cultures. As a "preincubation" step, incubation medium (1.5 mL) that contained either troleandomycin, LY335979, or an appropriate vehicle was added to the apical (luminal) chamber of the insert, followed by an equal volume of plain incubation medium to the basolateral chamber. After 30 minutes at 37°C, apical and basolateral media were removed and immediately replaced with the various combinations of erythromycin or digoxin and preincubation compound or vehicle. Final concentrations of troleandomycin, LY335979, dimethyl sulfoxide, and ethanol were 30 μmol/L, 0.5 μmol/L, 0.1% (vol/vol), and 1% (vol/vol), respectively. Under these conditions, troleandomycin was at the limit of solubility.

To evaluate apical-to-basolateral (A$\rightarrow$B) translocation of erythromycin across the cell monolayers, incubation medium that contained [^{14}C]erythromycin (10 μmol/L, 0.5 μCi) and one of the preincubation compounds (or vehicle) was added to the apical chamber, followed by incubation medium plus an appropriate vehicle to the basolateral chamber. To evaluate basolateral-to-apical (B$\rightarrow$A) translocation of erythromycin, incubation medium that contained [^{14}C]-erythromycin was added to the basolateral chamber, followed by incubation medium that contained one of the preincubation compounds (or vehicle) to the apical chamber. Samples (40 μL) were collected from both the apical and basolateral compartments of each insert at 1, 2, 3, and 4 hours after the addition of [^{14}C]erythromycin. As a positive control for P-glycoprotein activity, an identical experiment was performed with the P-glycoprotein substrate [^{3}H]digoxin (5 μmol/L, 1 μCi). The medium collected from the apical and basolateral compartments was analyzed by liquid scintillation counting. The percentage of [^{14}C]erythromycin or [^{3}H]digoxin translocated to the receiving chamber was calculated as the ratio of radioactivity measured in the

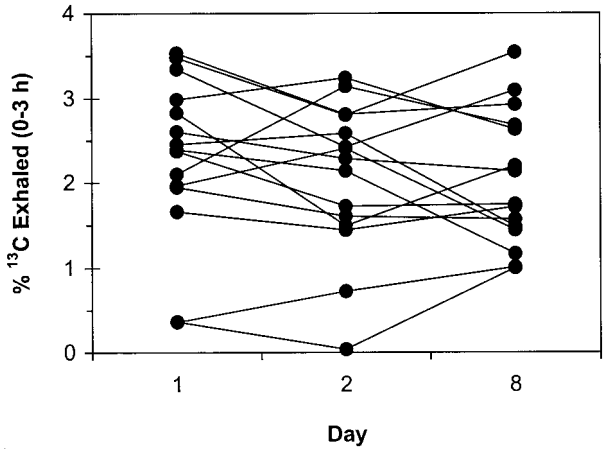

Fig 1. Intrasubject reproducibility in 15 healthy volunteers of the result for the oral stable isotope (^{13}C)-labeled formulation of the erythromycin breath test, expressed as the percentage of the erythromycin dose (500 mg) exhaled as $^{13}CO_2$ over the 0- to 3-hour collection interval.

receiving chamber to the sum of radioactivity measured in the dosing and receiving chambers. Efflux ratios—measures of net secretion—were calculated as the ratio of the percentage of radiolabeled drug translocated into the basolateral chamber to that translocated into the apical chamber during the 4-hour period.

Statistical analysis

All statistical analyses were performed with use of StatView (version 5.0.1; SAS Institute Inc, Cary, NC). For the human subject study, nonparametric methods were used because the data were not assumed to be normally distributed. The Wilcoxon signed-rank test was used to determine whether the effects of troleandomycin or rifampin treatment on the various ERMBT and pharmacokinetic measurements were significantly different from baseline (significance level of .05). Relationships between the $ERMBT_{IV}$ and $ERMBT_{oral}$ results and between each ERMBT result and erythromycin CL/F or C_{max} were assessed from the Spearman correlation coefficient (r_S), which was considered to be significant if the P value was $<.05$. For the cell culture studies, the unpaired t test with equal variance was used to determine whether a difference existed between vehicle-treated and troleandomycin- or LY335979-treated culture inserts (significance level of .05).

RESULTS

Intrasubject variation in the $ERMBT_{oral}$

A preliminary investigation was conducted in 15 healthy volunteers to evaluate the interday variability in the $ERMBT_{oral}$ within a subject. Fig 1 shows the test result, expressed as the percentage of the dose oxidized during the 0- to 3-hour breath collection interval, for each subject on each of 3 days. Virtually identical patterns ensued if the test result were based on the 1- to 3- or 2- to 3-hour collection interval (data not shown). Within-subject variation was generally good (median, 19%; range, 9% to 105%). We therefore proceeded with our evaluation of the $ERMBT_{oral}$ in a separate group of healthy volunteers. Our objective was to compare the effects of the CYP3A4 modulators troleandomycin and rifampin on the $ERMBT_{IV}$ result, the $ERMBT_{oral}$ result, and on oral erythromycin pharmacokinetics.

$ERMBT_{IV}$

In the current group of subjects (n = 14), the median $ERMBT_{IV}$ result at baseline was 1.85 and varied 3-fold (range, 1.03-2.97; Fig 2, *A*). After troleandomycin administration, the $ERMBT_{IV}$ result markedly decreased in all subjects; the median test result was 0.09 (range, 0.05-0.22) and was significantly lower than that at baseline ($P = .001$; Fig 2, *A*). After rifampin treatment, the $ERMBT_{IV}$ result increased in all subjects, from 1.4- to 5.7-fold above baseline (Fig 2, *A*). The median test result significantly increased from baseline (to 4.23; $P = .001$), and individual results ranged from 2.49 to 5.87.

$ERMBT_{oral}$

At baseline, the median (1.91) and range (0.63-2.66) in the $ERMBT_{oral}$ result, expressed as the percentage of $^{13}CO_2$ exhaled during the 0- to 3-hour interval (Fig 2, *B*), were comparable with those observed in our preliminary investigation of 15 healthy subjects (Fig 1). After troleandomycin administration, as with the $ERMBT_{IV}$ result, the $ERMBT_{oral}$ result decreased in all subjects, and the median value (0.57; range, 0.00-0.83) was significantly lower than that at baseline ($P = .001$; Fig 2, *B*). After rifampin treatment, unlike with the $ERMBT_{IV}$ result, the $ERMBT_{oral}$ result increased ($\geq$1.3-fold above baseline) in only half of the subjects, and no more than 1.7-fold (Fig 2, *B*). For the remaining subjects, the oral test result was unchanged or was even decreased (by as much as 90%) from baseline. Consequently, the median $ERMBT_{oral}$ result (1.97; range, 0.20-3.69) was not significantly different from that at baseline ($P = .30$).

Oral erythromycin pharmacokinetics

Two representative plasma concentration versus time profiles for erythromycin after administration of the

$ERMBT_{oral}$ for all 3 phases of the study are shown in Fig 3, *A* and *C,* in which the rank order of AUC values (troleandomycin > baseline > rifampin) was readily apparent. Table I displays the median values and ranges of the various pharmacokinetic measures (t_{max}, C_{max}, λ_Z, and CL/F) determined for each phase of the study, and those at baseline agreed with previous reports.[21,22] The C_{max} was generally achieved within 1.5 to 2.5 hours and, compared with baseline, the median t_{max} was unchanged after troleandomycin ($P = .19$) and rifampin ($P = .37$) treatment. Compared with baseline, the median C_{max} significantly increased with troleandomycin treatment (1.34 versus 2.92 mg/L; $P = .001$) and significantly decreased with rifampin treatment (to 0.72 mg/L; $P = .04$). The median λ_Z significantly decreased from baseline with troleandomycin treatment (0.35 versus 0.24 h^{-1}; $P = .006$) but remained unchanged after rifampin treatment (0.38 h^{-1}; $P = .70$). These values corresponded to terminal $t_{1/2}$ values of 2.0 hours (baseline), 2.9 hours (troleandomycin), and 1.8 hours (rifampin). The percentage of total AUC extrapolated from 10 hours to infinite time was always <30%. The median erythromycin oral clearance displayed a pattern similar to the $ERMBT_{IV}$ result; that is, CL/F decreased significantly ($P = .001$), by more than 70% compared with baseline, after troleandomycin administration (96 versus 26 L/h) and significantly increased ($P = .013$) after rifampin treatment (to 197 L/h). There was no change in the variability in erythromycin CL/F when adjusted for body weight for all 3 phases of the study (data not shown).

Corresponding representative $ERMBT_{oral}$ profiles (percentage of ^{13}C exhaled versus time) are presented in Fig 3, *B* and *D*. For the subject shown in Fig 3, *B,* the rank order of breath AUC values (rifampin > baseline > troleandomycin) was opposite that for plasma AUC values (Fig 3, *A*). This would be expected if the reduction and elevation in erythromycin plasma levels resulted solely from induction and inhibition, respectively, of CYP3A4. For the subject shown in Fig 3, *D,* however, the rank order of breath AUC values (baseline > troleandomycin > rifampin) was not opposite that for plasma AUC values (Fig 3, *C*). This paradoxic scenario occurred in half of the 14 subjects.

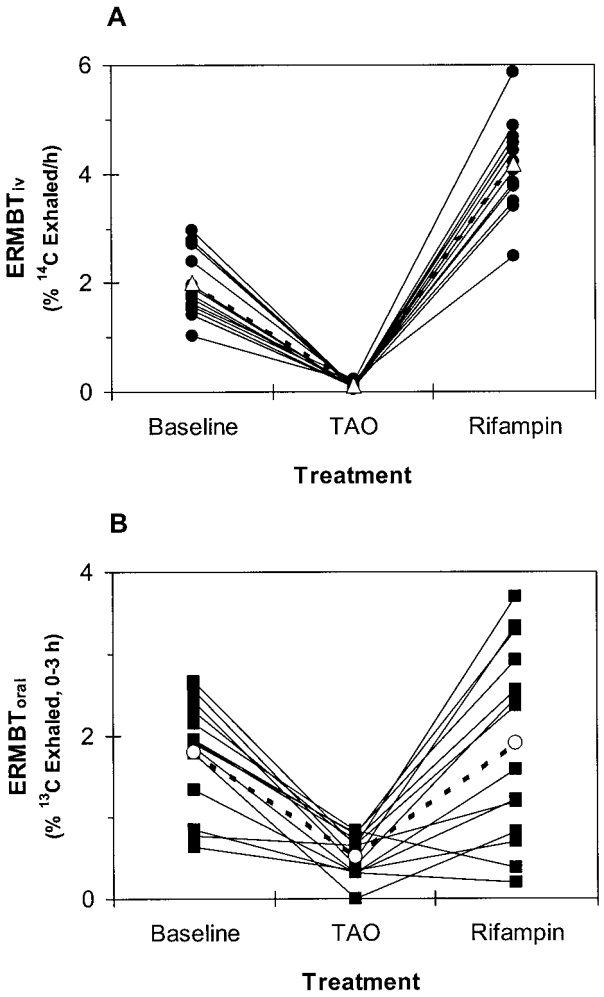

Fig 2. Effects of troleandomycin (TAO) treatment (single dose of 500 mg) and rifampin treatment (600 mg daily for 7 days) on the intravenous ^{14}C-erythromycin breath test ($ERMBT_{IV}$) result **(A)** and the oral stable isotope (^{13}C)-labeled formulation of the erythromycin breath test ($ERMBT_{oral}$) result **(B)** in 14 healthy volunteers. *Open symbols* and *broken lines* denote median results.

Correlation analyses

Although the $ERMBT_{IV}$ and $ERMBT_{oral}$ results showed similar trends at baseline and with troleandomycin treatment, there was no rank-order correlation between the 2 test results within either treatment group ($r_S = 0.47$ and $r_S = -0.18$, respectively; $P \geq .09$). Not surprisingly, there was no association between the 2 ERMBT results within the rifampin phase ($r_S = -0.002$; $P = .99$). There were no correlations between erythromycin CL/F and either ERMBT result or between erythromycin C_{max} and the $ERMBT_{IV}$ result within each treatment group ($P \geq .05$). For the association between erythromycin C_{max} and the $ERMBT_{oral}$ result, there was a positive but insignificant trend at baseline ($r_S = 0.53$; $P = .06$), no correlation with troleandomycin treatment ($r_S = -0.23$; $P = .41$), and a significant correlation with rifampin treatment ($r_S = 0.64$; $P = .02$; Fig 4).

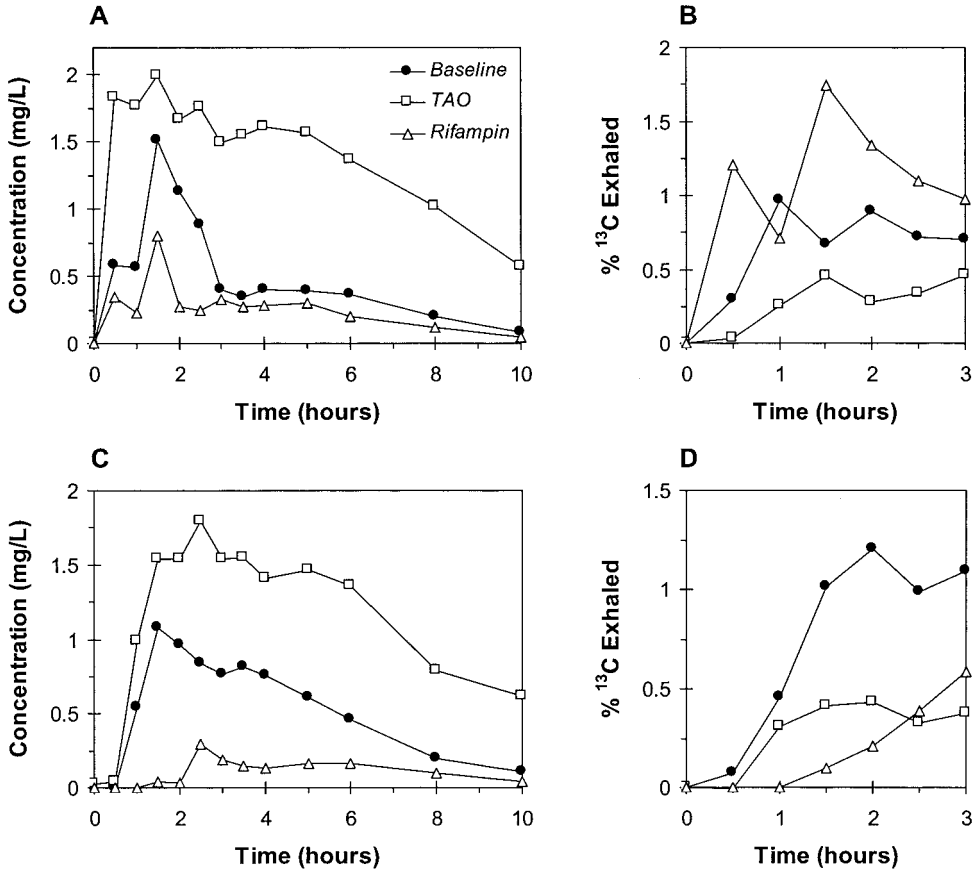

Fig 3. Representative erythromycin plasma concentration–versus–time (**A** and **C**) and corresponding $^{13}CO_2$-labeled breath–versus–time (**B** and **D**) profiles. The rank-order area under the curve for the breath test profile was either opposite **(B)** or not opposite **(D)** its plasma–time profile counterpart. TAO, Troleandomycin.

Troleandomycin is not an inhibitor of erythromycin transport

To determine whether troleandomycin may have confounded the interpretation of our in vivo results (by altering erythromycin transport), we conducted an in vitro experiment using [^{14}C-*N*-methyl]erythromycin and the human intestinal cell line Caco-2, which is known to express P-glycoprotein (and other transporters). As a positive control for P-glycoprotein activity, an identical experiment was conducted with use of the established P-glycoprotein probe substrate digoxin.[23,24] For all culture inserts, at least 90% of the initial amount of radioactivity added was recovered in the media collected from the apical and basolateral chambers at the end of the 4-hour incubation. Compared with vehicle-treated culture inserts, troleandomycin had no effect on the mean percentage of erythromycin translocated in both the A→B direction (0.5% versus 0.5%; $P = .34$) and B→A direction (48.9% versus 47.0%; $P = .20$; Fig 5, *A* and *B*). In contrast, the selective P-glycoprotein inhibitor LY335979 significantly increased mean A→B translocation of erythromycin (to 6.4%; $P < .0001$) and decreased mean B→A translocation of erythromycin (to 12.5%; $P < .0001$; Fig 5, *C*). Similarly, compared with vehicle-treated cultures, the average efflux ratio of erythromycin was unaffected by troleandomycin (99.8 versus 87.9; $P = .11$) but was significantly decreased by LY335979 (to 1.96; $P < .0001$). Results with digoxin-treated cultures (Fig 5, *D, E,* and *F*) were qualitatively identical to erythromycin-treated cultures with statistically similar trends. Compared with erythromycin, the mean B→A translocation for digoxin was similar (43.4% and 44.6% for vehicle- and troleandomycin-treated cultures, respectively), whereas the mean A→B translocation was 5- to 7-fold greater (3.4% and 2.5% for vehicle- and

Table I. Pharmacokinetics of erythromycin (500 mg) after oral administration to 14 healthy volunteers

Study phase	C_{max} *(mg/L)*	t_{max} *(h)*	λ_Z (h^{-1})	*CL/F (L/h)*
Baseline				
Median	1.34	1.5	0.35	96
Range	0.40-3.16	0.5-5.0	0.21-0.45	37-250
Troleandomycin				
Median	2.92*	2.0	0.24*	26*
Range	0.95-4.80	1.0-5.0	0.15-0.38	17-73
Rifampin (INN, rifampicin)				
Median	0.72*	2.5	0.38	197*
Range	0.06-1.66	1.0-5.0	0.14-0.51	102-2015

C_{max}, Maximum plasma concentration; t_{max}, time to reach C_{max}; λ_Z, terminal elimination rate constant; CL/F, apparent oral clearance.
*Statistically different from baseline ($P < .05$; Wilcoxon signed-rank test).

troleandomycin-treated cultures, respectively). Consequently, the mean efflux ratio for digoxin was roughly 5-fold lower (15.5 and 19.5 for vehicle- and troleandomycin-treated cultures, respectively) than that for erythromycin. LY335979 inhibited both A→B and B→A digoxin translocation so that the percentage of the dose translocated in each direction was similar to that for erythromycin (8.7% and 12.1% for A→B and B→A, respectively), yielding an efflux ratio (1.44) similar to that for erythromycin.

DISCUSSION

The overall goal of our study was to develop an oral erythromycin breath test that was capable of assessing aggregate liver and intestinal CYP3A4 activity. Analogous to the $ERMBT_{IV}$, and with no diet restrictions, the oral ^{13}C-labeled formulation displayed good intrasubject reproducibility for percentage of $^{13}CO_2$ exhaled in breath during the 0- to 3-hour interval after administration (Fig 1). The larger values for interday variation observed in the 2 subjects with the lowest values (<0.5%) were attributed to the greater sensitivity of low values to the natural fluctuations in the ratio of ^{13}C to ^{12}C in expired CO_2, which can be influenced by food and exercise.[25] Accordingly, we proceeded with our evaluation of this new test, which was to compare the effects of prototypical CYP3A4 modulators on the $ERMBT_{oral}$ result, on the $ERMBT_{IV}$ result, and on oral erythromycin disposition.

As expected, pretreatment with a single oral dose of the CYP3A4 mechanism-based inhibitor troleandomycin significantly decreased the median rate of labeled CO_2 production relative to baseline, regardless of the route of erythromycin administration. Compared with intravenous administration, the apparent weaker degree of inhibition of erythromycin demethylation after oral administration (95% versus 70%) may have arisen from the time course of troleandomycin inhibition. With the $ERMBT_{IV}$, a single breath sample was collected 20 minutes after injection, or 2 hours and 20 minutes after troleandomycin was given. With the $ERMBT_{oral}$, breath samples were continually collected until 3 hours after drug administration, or 5 hours after troleandomycin was given. With the $ERMBT_{IV}$ we have found that inhibition of CYP3A4 by a single oral dose of troleandomycin peaks between 2 and 3 hours, then wanes thereafter (unpublished observations). The reduced magnitude of inhibition observed with the $ERMBT_{oral}$ may therefore simply have reflected a "wearing off" of the troleandomycin effect. As with the ERMBTs, troleandomycin treatment significantly decreased the median CL/F of erythromycin (to less than one third of that at baseline), consistent with the premise that troleandomycin interacts with erythromycin by inhibition of CYP3A4, which could occur in the intestine or liver.

Rifampin treatment doubled the median rate of $^{14}CO_2$ exhaled from the $ERMBT_{IV}$, which was anticipated because rifampin is a well-established and potent inducer of CYP3A4. Likewise, the median CL/F of erythromycin doubled and, together with the $ERMBT_{IV}$ result, was consistent with hepatic CYP3A4 induction as a mechanism that underlies the oral erythromycin-rifampin interaction. Unlike troleandomycin, however, rifampin did not alter the median production of $^{13}CO_2$ from the $ERMBT_{oral}$. That is, in spite of a marked interaction between rifampin and oral erythromycin, the total demethylation of erythromycin unexpectedly appeared to be unchanged relative to baseline. One explanation is that rifampin treatment indeed resulted in increased erythromycin *N*-demethylation in the intestine, but this was not reflected in increased production of breath $^{13}CO_2$. This could occur if the ^{13}C-labeled formaldehyde generated in the intestine did not undergo the same fate (in terms of one carbon pool kinetics) as

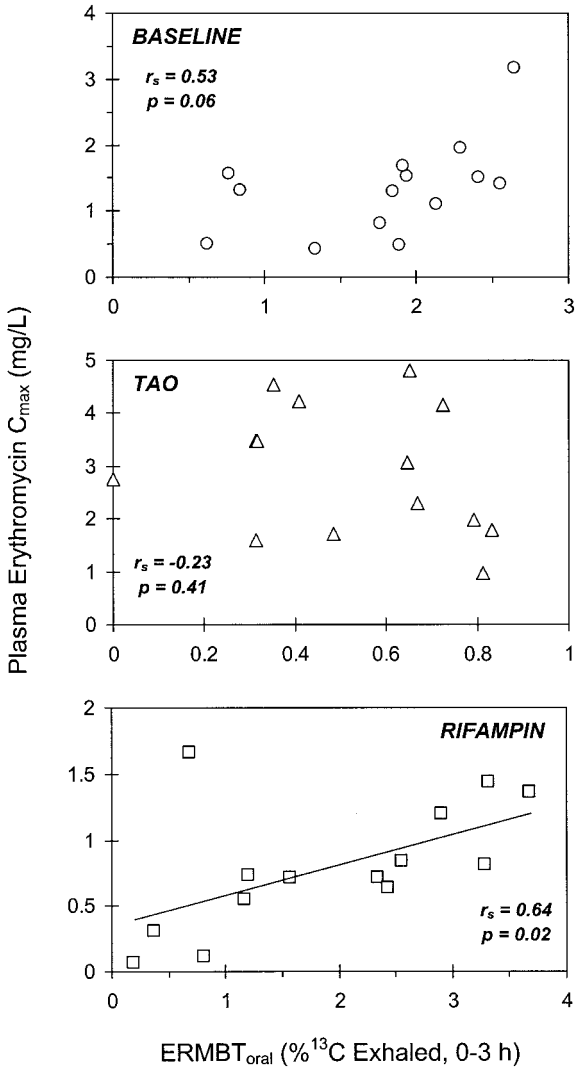

Fig 4. Spearman rank correlation between the $ERMBT_{oral}$ result and plasma erythromycin maximum plasma concentration (C_{max}) at baseline after troleandomycin (TAO) treatment (single dose of 500 mg) and after rifampin treatment (600 mg daily for 7 days) in 14 healthy subjects.

formaldehyde generated in the liver. This seems to be unlikely, however, because the enzymes involved in one carbon pool kinetics are believed to be ubiquitous.

Another unforeseen finding was the positive association between erythromycin C_{max} and the $ERMBT_{oral}$ result at baseline and with rifampin treatment (Fig 4). If erythromycin C_{max} were assumed to be largely a reflection of first-pass metabolism, an association between a high rate of $^{13}CO_2$ production and a low C_{max} would be expected. However, the opposite trend occurred (a high rate of $^{13}CO_2$ production was associated with a high C_{max}), indicating that CYP3A4-mediated first-pass metabolism (in the intestine or liver) is not the major determinant of erythromycin C_{max}, particularly during rifampin treatment. Collectively, the lack of effect of rifampin on the median rate of erythromycin demethylation (as indicated by the $ERMBT_{oral}$) and the positive association between erythromycin C_{max} and the $ERMBT_{oral}$ result strongly imply that a mechanism(s) in addition to CYP3A4 induction must account for the oral erythromycin-rifampin interaction. One plausible mechanism may involve the transmembrane secretory pump P-glycoprotein.

P-glycoprotein, the product of the multidrug resistance gene *MDR1,* is an apically directed and adenosine triphosphate–dependent transporter that was first identified in human cancer cells; it acts to extrude certain chemotherapeutic agents from the cell and to render the agent ineffective.[26] P-glycoprotein has also been detected in normal human tissues, including capillary endothelial cells of the brain, epithelial cells (brush border) of the renal tubule and intestine, and the canalicular membrane of hepatocytes.[26,27] Because of its luminal-canalicular location in these tissues, P-glycoprotein functions to limit brain uptake, to reduce absorption, and to enhance the renal, intestinal, and biliary excretion of its substrates (reviewed by Ambudkar et al[26]). Along with numerous other drugs and xenobiotics, erythromycin has been shown to be a substrate for P-glycoprotein, and a pronounced role for this efflux pump in erythromycin disposition in vivo in rats has been reported.[28]

In humans, rifampin is an inducer not only of intestinal (and hepatic) CYP3A4 but also of intestinal P-glycoprotein.[24,29] Although not confirmed by direct intestinal biopsy measurements in this study, we can assume that rifampin treatment induced both proteins. Consequently, the reduction in oral erythromycin AUC by rifampin was likely largely the result of an increase in intestinal P-glycoprotein levels. The lack of effect of rifampin on the median $ERMBT_{oral}$ result could therefore be explained if induction of P-glycoprotein resulted in roughly a 50% decrease in the median amount of erythromycin absorbed from the gut lumen. This alone would explain why the median AUC value of erythromycin after rifampin administration was approximately half of the median erythromycin AUC at baseline (2.5 versus 5.2 mg · h/L). A 2-fold induction in CYP3A4 activity (as reflected in the 2-fold increase in the median $ERMBT_{IV}$ result) would therefore explain why the median $ERMBT_{oral}$ result was similar to that observed at baseline. In other words, the median rate of $^{13}CO_2$ expired from the $ERMBT_{oral}$ remained un-

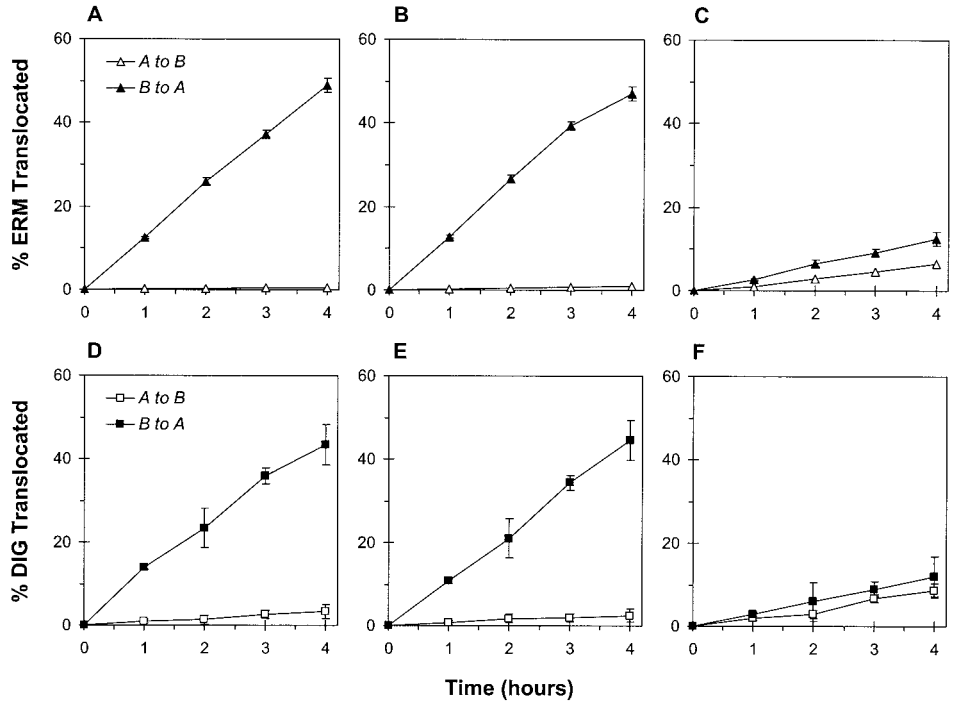

Fig 5. Apical-to-basolateral (A to B) and basolateral-to-apical (B to A) translocation of [^{14}C]-erythromycin (10 μmol/L, 0.5 μCi; **A, B,** and **C**) and [^{3}H]digoxin (5 μmol/L, 1 μCi; **D, E,** and **F**) in Caco-2 cell monolayers. Cell monolayers were incubated with vehicle (**A** and **D**), the CYP3A4 inhibitor troleandomycin (30 μmol/L; **B** and **E**), or the selective P-glycoprotein inhibitor LY335979 (0.5 μmol/L; **C** and **F**) alone for 30 minutes and then with erythromycin (ERM) or digoxin (DIG) plus vehicle or inhibitor for 4 hours. *Symbols* and *error bars* denote mean values and standard deviations, respectively, of triplicate culture inserts.

changed after rifampin treatment because CYP3A4 induction (in the intestine and liver) was offset by the reduced delivery of erythromycin to the enzyme (because of reduced absorption).

P-glycoprotein in organs other than the intestine (eg, kidney, brain, and liver) may have also influenced erythromycin disposition. However, the percentage of an oral erythromycin dose excreted unchanged in the urine (2% to 5%)[30] is minimal, making a role for renal P-glycoprotein unlikely. Although erythromycin is not detected in normal cerebrospinal fluid and brain tissue,[30] perhaps suggesting a role for cerebral P-glycoprotein, it seems to be intuitively unlikely that P-glycoprotein in the central nervous system would have a major impact on erythromycin disposition. A role for hepatic P-glycoprotein, however, cannot be ruled out. When *mdr1a* nullizygous (−/−) and *mdr1a/1b* (−/−) mice received an intravenous injection of [^{14}C-*N*-methyl]erythromycin, the breath $^{14}CO_2$ AUC was 1.5- and 1.9-fold greater, respectively, compared with that for the wild-type counterparts, despite having equivalent levels of hepatic CYP3A4.[31] These observations raised the possibility that hepatic P-glycoprotein influenced CYP3A4-mediated metabolism. Although induction of hepatic P-glycoprotein by rifampin may have occurred and may have influenced the current observations, it would be expected that such induction would have comparable effects on both the oral and $ERMBT_{IV}$ results. Hepatic P-glycoprotein therefore would not account for the discrepancy between the $ERMBT_{oral}$ and $ERMBT_{IV}$ results.

Troleandomycin did not alter the transport of erythromycin or of the P-glycoprotein substrate digoxin in monolayers of the human intestinal cell line Caco-2, suggesting that the effects observed in vivo with troleandomycin pretreatment were the result of selective CYP3A4 inhibition. We therefore concluded that CYP3A4-mediated metabolism represents a major pathway for the elimination of erythromycin in uninduced individuals. The lack of effect of troleandomycin

on P-glycoprotein activity also explained why the changes in the $ERMBT_{IV}$ result, the $ERMBT_{oral}$ result, and the various oral erythromycin pharmacokinetic measurements were the same as the predicted changes.

Rivory et al[32] recently reported that the $ERMBT_{IV}$ result did not correlate with the systemic clearance of erythromycin in 16 adult patients with cancer. Although we did not directly assess the systemic clearance of erythromycin in this study, it is interesting that the median λ_Z of erythromycin was only modestly reduced after CYP3A4 inhibition (by troleandomycin) and was unaffected after CYP3A4 induction (by rifampin; Table I). Assuming that the 2 modulators of CYP3A4 did not alter the (terminal) volume of distribution of erythromycin, these observations support the contention that CYP3A4-mediated metabolism is not rate limiting in the systemic clearance of erythromycin. It should be noted that the contribution of other non–CYP3A4-mediated pathways in the systemic clearance of erythromycin would not invalidate the $ERMBT_{IV}$ as a measure of hepatic CYP3A4 activity, unless such pathways significantly altered the hepatic concentration of erythromycin 20 minutes after injection (ie, when the single breath sample is collected).[33]

In conclusion, because the oral erythromycin breath test does not appear to simply reflect aggregate intestinal and hepatic CYP3A4 activity, this test would not be suitable as an in vivo CYP3A4 phenotyping probe. However, a key finding during the course of the evaluation of this test was that the oral erythromycin-rifampin interaction was not solely the result of CYP3A4 induction and likely also reflected intestinal P-glycoprotein induction. This would not have surfaced if we had examined only erythromycin pharmacokinetics. To our knowledge, the erythromycin-rifampin interaction represents the first example in which P-glycoprotein appears to be a major locus for an interaction that involves a substrate administered in half-gram quantities. It therefore seems that P-glycoprotein can serve as a relatively high-capacity xenobiotic transporter in vivo. Although erythromycin and rifampin are rarely prescribed together, results from this study may well apply to drug-rifampin interactions that involve other CYP3A4 and P-glycoprotein substrates similarly given in large quantities (eg, the human immunodeficiency virus protease inhibitors saquinavir, indinavir, and amprenavir). What may appear to be consistent with CYP3A4-mediated metabolism may also reflect P-glycoprotein-mediated efflux.

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References

1. Watkins PB, Murray SA, Winkelman LG, Heuman DM, Wrighton SA, Guzelian PS. Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochromes P-450: studies in rats and patients. J Clin Invest 1989; 83:688-97.
2. Baker AL, Kotake AN, Schoeller DA. Clinical utility of breath tests for the assessment of hepatic function. Semin Liver Dis 1983;3:318-29.
3. Rivory LP, Slaviero KA, Hoskins JM, Clarke SJ. The erythromycin breath test for the prediction of drug clearance. Clin Pharmacokinet 2001;40:151-8.
4. Watkins PB. Noninvasive tests of CYP3A enzymes. Pharmacogenetics 1994;4:171-84.
5. Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, Schmiedlin-Ren P, et al. Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. Clin Pharmacol Ther 1997;62:248-60.
6. Cheng CL, Smith DE, Carver PL, Cox SR, Watkins PB, Blake DS, et al. Steady-state pharmacokinetics of delavirdine in HIV-positive patients: effect on erythromycin breath test [see comments]. Clin Pharmacol Ther 1997; 61:531-43.
7. Hirth J, Watkins PB, Strawderman M, Schott A, Bruno R, Baker LH. The effect of an individual's cytochrome CYP3A4 activity on docetaxel clearance [see comments]. Clin Cancer Res 2000;6:1255-8.
8. Gharaibeh MN, Gillen LP, Osborne B, Schwartz JI, Waldman SA. Effect of multiple doses of rifampin on the [^{14}C-*N*-methyl] erythromycin breath test in healthy male volunteers. J Clin Pharmacol 1998;38:492-5.
9. McCune JS, Hawke RL, LeCluyse EL, Gillenwater HH, Hamilton G, Ritchie J, et al. In vivo and in vitro induction of human cytochrome P4503A4 by dexamethasone. Clin Pharmacol Ther 2000;68:356-66.
10. Jamis-Dow CA, Pearl ML, Watkins PB, Blake DS, Klecker RW, Collins JM. Predicting drug interactions in vivo from experiments in vitro: human studies with paclitaxel and ketoconazole. Am J Clin Oncol 1997;20: 592-9.
11. Brophy DF, Israel DS, Pastor A, Gillotin C, Chittick GE, Symonds WT, et al. Pharmacokinetic interaction between amprenavir and clarithromycin in healthy male volunteers. Antimicrob Agents Chemother 2000;44:978-84.
12. Polk RE, Crouch MA, Israel DS, Pastor A, Sadler BM, Chittick GE, et al. Pharmacokinetic interaction between ketoconazole and amprenavir after single doses in healthy men. Pharmacotherapy 1999;19:1378-84.
13. Kolars JC, Schmiedlin-Ren P, Schuetz JD, Fang C, Watkins PB. Identification of rifampin-inducible P450IIIA4 (CYP3A4) in human small bowel enterocytes. J Clin Invest 1992;90:1871-8.
14. Lown K, Kolars J, Turgeon K, Merion R, Wrighton SA, Watkins PB. The erythromycin breath test selectively

measures P450IIIA in patients with severe liver disease. Clin Pharmacol Ther 1992;51:229-38.
15. Watkins PB, Turgeon DK, Saenger P, Lown KS, Kolars JC, Hamilton T, et al. Comparison of urinary 6-β-cortisol and the erythromycin breath test as measures of hepatic P450IIIA (CYP3A) activity. Clin Pharmacol Ther 1992; 52:265-73.
16. Dantzig AH, Shepard RL, Law KL, Tabas L, Pratt S, Gillespie JS, et al. Selectivity of the multidrug resistance modulator, LY335979, for P-glycoprotein and effect on cytochrome P-450 activities. J Pharmacol Exp Ther 1999;290:854-62.
17. Wagner D. CYP3A4 and the erythromycin breath test [letter]. Clin Pharmacol Ther 1998;64:129-30.
18. Shreeve WW, Shoop JD, Ott DG, McInteer BB. Test for alcoholic cirrhosis by conversion of [^{14}C]- or [^{13}C]galactose to expired CO_2. Gastroenterology 1976;71:98-101.
19. Fisher JM, Wrighton SA, Watkins PB, Schmiedlin-Ren P, Calamia JC, Shen DD, et al. First-pass midazolam metabolism catalyzed by 1α,25-dihydroxy vitamin D_3-modified Caco-2 cell monolayers. J Pharmacol Exp Ther 1999;289:1134-42.
20. Schmiedlin-Ren P, Thummel KE, Fisher JM, Paine MF, Lown KS, Watkins PB. Expression of enzymatically active CYP3A4 by Caco-2 cells grown on extracellular matrix-coated permeable supports in the presence of 1α,25-dihydroxyvitamin D_3. Mol Pharmacol 1997;51: 741-54.
21. Hall KW, Nightingale CH, Gibaldi M, Nelson E, Bates TR, DiSanto AR. Pharmacokinetics of erythromycin in normal and alcoholic liver disease subjects. J Clin Pharmacol 1982;22:321-5.
22. Birkett DJ, Robson RA, Grgurinovich N, Tonkin A. Single oral dose pharmacokinetics of erythromycin and roxithromycin and the effects of chronic dosing. Ther Drug Monit 1990;12:65-71.
23. Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P. Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J Clin Invest 1995;96:1698-705.
24. Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, Zundler J, et al. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. J Clin Invest 1999;104:147-53.
25. Schoeller DA, Schneider JF, Solomons NW, Watkins JB, Klein PD. Clinical diagnosis with the stable isotope ^{13}C in CO_2 breath tests: methodology and fundamental considerations. J Lab Clin Med 1977;90:412-21.
26. Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol 1999;39:361-98.
27. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci U S A 1987;84: 7735-8.
28. Schuetz EG, Yasuda K, Arimori K, Schuetz JD. Human MDR1 and mouse mdr1a P-glycoprotein alter the cellular retention and disposition of erythromycin, but not of retinoic acid or benzo(a)pyrene. Arch Biochem Biophys 1998;350:340-7.
29. Westphal K, Weinbrenner A, Zschiesche M, Franke G, Knoke M, Oertel R, et al. Induction of P-glycoprotein by rifampin increases intestinal secretion of talinolol in human beings: a new type of drug/drug interaction. Clin Pharmacol Ther 2000;68:345-55.
30. Kapusnick-Uner JE, Sande MA, Chambers HF. Antimicrobial agents: tetracyclines, chloramphenicol, erythromycin, and miscellaneous antibacterial agents. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman AG, editors. Goodman and Gilman's the pharmacological basis of therapeutics. 9th ed. New York: McGraw-Hill; 1996. p. 1123-53.
31. Lan LB, Dalton JT, Schuetz EG. Mdr1 limits CYP3A metabolism in vivo. Mol Pharmacol 2000;58:863-9.
32. Rivory LP, Slaviero K, Seale JP, Hoskins JM, Boyer M, Beale PJ, et al. Optimizing the erythromycin breath test for use in cancer patients. Clin Cancer Res 2000;6: 3480-5.
33. Rivory LP, Watkins PB. Erythromycin breath test [letter]. Clin Pharmacol Ther 2001;70:395-7.