Measurement of hepatic and intestinal CYP3A4 and PGP activity by combined po + iv $[^{14}\text{C}]$erythromycin breath and urine test

W. P. D. Lemahieu, B. D. Maes, Y. Ghoos, P. Rutgeerts, K. Verbeke, and Y. Vanrenterghem

Department of Medicine, Division of Nephrology and Gastrointestinal Research Center, University Hospital Gasthuisberg, Leuven B-3000, Belgium

Submitted 16 January 2003; accepted in final form 12 May 2003

Lemahieu, W. P. D., B. D. Maes, Y. Ghoos, P. Rutgeerts, K. Verbeke, and Y. Vanrenterghem. Measurement of hepatic and intestinal CYP3A4 and PGP activity by combined po + iv $[^{14}\text{C}]$erythromycin breath and urine test. Am J Physiol Gastrointest Liver Physiol 285: G470–G482, 2003.—The aim of the present study was to develop and validate a combined breath and urine test with $[^{14}\text{C}]$erythromycin for measurement of both hepatic and intestinal CYP3A4 and PGP activity in vivo in human clinical practice.

The rationale of the test was based on the fact that erythromycin is a probe for both CYP3A4 and PGP (1, 20). We hypothesized that dynamic $^{14}\text{CO}_2$ excretion measurements in breath after both iv and oral (po) administration of $[^{14}\text{C}]$erythromycin in addition to mathematical deconvolution (34) of the resulting curves would allow to evaluate both hepatic and intestinal CYP3A4 activity separately. $^{14}\text{C}$-labeled excretion of the iv and po administered tracer in the urine reflects the portion of $[^{14}\text{C}]$erythromycin that has escaped the excretory activity of PGP. In this way, it is inversely related to the activity of hepatic and intestinal PGP. With the use of the same mathematical deconvolution model, hepatic and intestinal...
PGP activity could theoretically be separated from each other. These hypotheses were tested in normal healthy volunteers in basal conditions and after pharmacological modulation and in renal transplant recipients early after transplantation.

**MATERIALS AND METHODS**

**Materials**

All tests were performed after an overnight fast. In the iv [14C]erythromycin breath and urine test (iv EBT + EUT), 74 kBq [N-methyl-14C]erythromycin (New England Nuclear Life Science Products, Boston, MA) were dissolved in 2 ml saline 0.9% immediately before testing and injected as a bolus in a cubital vein via a 0.2-μm pore biofilter (Pall, Ann Arbor, MI). In the po [14C]erythromycin breath and urine test (po EBT + EUT), 74 kBq of [N-methyl-14C]erythromycin were incorporated in a lactose-containing gelatin capsule (Belgica T.O.P., Turnhout, Belgium). The capsule was ingested with 150 ml of water.

The iv and po EBT + EUT were always performed on two consecutive days in the same order (first iv, then po). Immediately before the tracer was injected/ingested, the urine bladder was emptied for a blanc urine sample and a blanc breath sample was taken. Breath samples were further taken every 10 min after injection/ingestion during 4 h. Urine collections were performed at 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h after injection/ingestion of the tracer. The subjects were kept fasting until the breath test was finished but were stimulated to drink water (750 ml) to make urine collections more reliable.

**Measuring Techniques**

Breath samples were obtained by exhaling breath through a pipette into a scintillation vial containing 2 mmol of hyamine hydroxide dissolved in 2 ml ethanol in the presence of thymolphthalein as indicator. Blowing was continued until the indicator decolorized, corresponding with the capture of 2 mmol of N-methyl-14C]erythromycin breath and urine test (iv EBT).

Measuring Techniques

**Materials**

All tests were performed after an overnight fast. In the iv [14C]erythromycin breath and urine test (iv EBT + EUT), 74 kBq [N-methyl-14C]erythromycin (New England Nuclear Life Science Products, Boston, MA) were dissolved in 2 ml saline 0.9% immediately before testing and injected as a bolus in a cubital vein via a 0.2-μm pore biofilter (Pall, Ann Arbor, MI). In the po [14C]erythromycin breath and urine test (po EBT + EUT), 74 kBq of [N-methyl-14C]erythromycin were incorporated in a lactose-containing gelatin capsule (Belgica T.O.P., Turnhout, Belgium). The capsule was ingested with 150 ml of water.

The iv and po EBT + EUT were always performed on two consecutive days in the same order (first iv, then po). Immediately before the tracer was injected/ingested, the urine bladder was emptied for a blanc urine sample and a blanc breath sample was taken. Breath samples were further taken every 10 min after injection/ingestion during 4 h. Urine collections were performed at 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h after injection/ingestion of the tracer. The subjects were kept fasting until the breath test was finished but were stimulated to drink water (750 ml) to make urine collections more reliable.

**Measuring Techniques**

Breath samples were obtained by exhaling breath through a pipette into a scintillation vial containing 2 mmol of hyamine hydroxide dissolved in 2 ml ethanol in the presence of thymolphthalein as indicator. Blowing was continued until the indicator decolorized, corresponding with the capture of 2 mmol of exhaled CO2. Next, 10 ml of scintillation cocktail (Hionic Fluor, Packard) were added, and radioactivity was measured by liquid scintillation spectrometry (Packard Tri-Carb Liquid Scintillation Spectrometer, model 3375; Packard Instruments, Dowsen Grove, IL). CO2 production was assumed to be 300 mmol·m⁻²·body surface area·h⁻¹. Body surface area was calculated using the weight-height formula of Haycock et al. (16). The results were expressed as the percentage of 14C recovery per hour of the given 14C dose (D) and as cumulative 14C dose curves (C) over 4 (EBT/EBT) EUT h. Detailed information of the technique used has been described in earlier reports (10). For the urine tests (EUT), only cumulative curves (C) were used due to the few measure points.

**Mathematical Analysis**

**Curve fitting.** The measured 14CO2 curves D and/or C in breath and urine were fitted according to the least square method by means of Excel visual basic software (Microsoft, Seattle, WA) by varying the parameters m, k, and β in

\[ C = m(1 - e^{-k\beta}) \]

for cumulative dose excretion curves (1)

\[ D = mk\beta e^{-k\beta}(1 - e^{-k\beta})^{-1} \]

for dose excretion curves, being the derivative of C (2) where \( t \) is the time in hours, and \( m, k, \) and \( \beta \) are constants, with \( m \) being the total cumulative 14C recovery when time is infinite.

This nonlinear regression analysis allows calculation of unequivocal parameters describing the processes such as excretion half-life (\( t_{1/2} \)) and excretion peak time (\( t_{\text{max}} \)); \( t_{1/2} = (-1/k) \times \ln(1 - 2^{-1/k}) \); \( t_{\text{max}} = \ln(2k)/k \). Substituting \( t_{\text{max}} \) in Eq. 2 also allows calculating a maximal excretion rate (\( D_{\text{max}} \)).

Mathematical separation of iv and po EBT and EUT. Because hepatic and intestinal CYP3A4 and PGP activity are part of a complex multicompartimental model, in which rate constants for intercompartmental exchange cannot be explicitly calculated, a previously reported separation model (34) was used to separate the hepatic and intestinal CYP3A4 and PGP activity as isolated functions by means of mathematical deconvolution.

Briefly, the iv and po EBT and EUT can be considered as convolution products of the processes involved as expressed in

\[ \text{iv EBT} = H_{\text{CYP3A}} \]  
\[ \text{po EBT} = I_{\text{CYP3A}} \times H_{\text{CYP3A}} \]  
\[ \text{iv EUT} = (1 - H_{\text{PGP}}) \times R \]  
\[ \text{po EUT} = (1 - I_{\text{PGP}}) \times (1 - H_{\text{PGP}}) \times R \]

in which \( H_{\text{CYP3A}} \) and \( I_{\text{CYP3A}} \) denote the hepatic and intestinal demethylatry process, respectively; \( H_{\text{PGP}} \) denotes the hepatic and \( I_{\text{PGP}} \) for the intestinal excretion process, and \( R \) denotes the renal excretion rate of absorbed, non-14C-labeled demethylated [N-methyl-14C]erythromycin that was not excreted by hepatic or intestinal PGP. From Eqs. 3 and 4, intestinal CYP3A4 catalytic activity can be isolated by applying the deconvolution technique to the iv and po EBT curves. Similarly, information on the hepatic and intestinal PGP activity can be obtained by application of the separation model to the iv (Eq. 5) and po (Eq. 6) EUT curves. The deconvolved curves were fitted, which allowed calculation of \( t_{1/2} \) and \( t_{\text{max}} \) of the deconvolved processes.

**Subjects**

Thirty-two healthy volunteers performed both an iv (day 1) and po (day 2) EBT and EUT in basal conditions after abstaining from smoking and consuming alcohol and/or grapefruit juice for at least 7 days before testing. None had a medical or surgical history or were taking drugs except for females in whom hormonal contraception was accepted as chronic drug intake. Twelve subjects repeated the test with washout periods of 7–10 days in pharmacologically modified test conditions: 1) after 200 mg of po itraconazole (Sporanox, Janssen-Cilag, Beerse, Belgium) twice a day (bid), the day before testing and 200 mg at the start of the test, 2) after 300 mg of po rifampicin (Rifadin; Aventis Pharma, Brussels, Belgium) bid during 5 days before testing and 300 mg at the start of the test, 3) after 200 ml of Seville orange juice bid the day before testing and 200 ml at the start of the test, and 4) after 200 ml of grapefruit juice (Tropicana red grapefruit juice, SA Looza NV, Borgloon, Belgium) bid the day before testing and 200 ml at the start of the test.

The different test conditions were applied in randomly assigned order, except for condition 2, which was always put as the final test condition because of the long-lasting effect of enzyme induction by rifampicin.

The po EBT and EUT in basal conditions were repeated three times within a 1-wk period in eight male subjects to evaluate the intraindividual variability of the test results.

To estimate the fate of [N-methyl-14C]erythromycin, fecal excretion of 14C was measured in seven subjects after po and
in five subjects after iv administration by daily collections of stools during 5 days.

To determine the possible influence of exposure to gastric acid on the capsule and its content used in the po EBT, seven subjects performed the po EBT and EUT at baseline and after intake of ranitidine 150 mg bid starting five days before and during testing. After a washout period of 1 wk, the same subjects also performed the test after 5 days intake of cimetidine 400 mg bid before and during testing. Also, the contact time of the capsule with the gastric (acid) environment was studied by measuring gastric emptying of the capsule in 12 healthy volunteers by the 13C-labeled octanoic acid breath test (34). This was done by incorporating 100 mg of 13C-labeled octanoic acid in the capsule. Additionally, gastric emptying of 200 ml of water was compared with that of 200 ml of grapefruit juice in six subjects to rule out possible influences of fruit juice on gastric emptying of the capsule.

Twenty-three patients were tested in basal conditions at day 7 or 8 after uncomplicated (no delayed graft function, no acute rejection) renal transplantation. Patients with a known history of gastrointestinal or metabolic diseases or with previous gastrointestinal surgery were excluded. They all received induction immunosuppressive therapy with corticosteroids and tacrolimus. Methylprednisolone (500 mg) was given intravenously 4 h before transplantation followed by 40 mg iv at day 1 and 20 mg po/day thereafter. Tacrolimus was started at 0.2 mg·kg⁻¹·day⁻¹ before transplantation and, thereafter, adjusted to obtain blood trough levels of 10–15 μg/l. All patients were concomitantly treated with cimetidine 400 mg/day, oral topical nystatin, and 800 mg/day trimethoprim-sulfamethoxazole. Fourteen patients were treated with po ganciclovir, 16 with anti-hypertensives (β₁-blocker 7, calcium entry blocker 10, ACE-I 5, ATII blocker 3), four with statins (atorvastatin 2, simvastatin 2), four with lorazepam, and three with allopurinol.

The Ethical Committee of the University of Leuven approved the study protocol. Written informed consent was obtained from all subjects.

Statistics

For statistical analyses, SAS software version 8.02 (SAS institute, Cary, NC) was used. P values <0.05 were considered significant.

---

Fig. 1. Median 14C recovery (in % of given dose) in breath and urine in 32 healthy volunteers after intravenous (iv; A and D) and oral (po; B and E) administration of 74 kBq of [14C]erythromycin and derived deconvolution curves (C and F). ◆, Measured data; solid line, fitted curve; light print, 14C recovery rate (%dose/h); heavy print, cumulative 14C recovery (%dose). EBT, erythromycin breath test; EUT, erythromycin urine test.
The relationship between calculated parameters of iv and po curves was analyzed by correlation analysis (SAS: PROC CORR). The test results obtained in healthy volunteers in different pharmacologically induced conditions were pairwise compared with those at baseline using the Wilcoxon’s signed rank test (SAS: PROC UNIVARIATE). Dunnett’s adjustment for multiple comparisons was made. The test results of healthy volunteers and patients were compared using the Wilcoxon’s rank sum test (SAS: PROC NPAR1WAY). Repeated-measures ANOVA (SAS: PROC GLM) and coefficient of variation (CV) were used to estimate the intraindividual (day to day) variability of the test results.

RESULTS

Healthy Volunteers: Baseline

After iv injection of 74 kBq of [N-methyl-14C]erythromycin (iv EBT; Fig. 1A), the percentage of 14C-labeled recovery in breath per hour of the given 14C dose (D) showed a steep ascending slope with a peak between 10 and 20 min, followed by an exponential descending limb of the 14CO2 excretion curve. The median %dose per hour recovery rate measured at 20 min (D20), identified by Turgeon et al. (48) as the most informative value to estimate hepatic CYP3A4 activity, was 2.57%/h. The cumulative 14C-dose curve (C) over 4 h has a total recovery of 14C of 5.12%. The calculated curve parameters resulted in an estimated median cumulative recovery at time infinite (m) of 6.24%, a t1/2 of 117 min, tmax of 12 min, and Dmax of 2.04%/h.

After po administration of 74 kBq of [N-methyl-14C]erythromycin (po EBT; Fig. 1B), the shape of the curves was clearly different: D had a slower ascending and descending slope with a lower peak excretion situated around 60 min [median %dose/h recovery at 60 min (D60): 0.23%/h] resulting in a flatter C curve with a median 14C-recovery at 4 h of 0.54%. This led to significantly altered calculated parameters of CYP3A4 activity and kinetics: Dmax lowered from 2.04 to 0.23%/h, m lowered to 0.64%, and tmax rose to 59 min; t1/2 did not change significantly.

Figure 1C shows the median D and C curve after deconvolution of the po and iv EBT curves (Fig. 1, A and B). These curves represent the percentage 14C recovery in breath (rate and total accumulation) of the fraction of tracer that eventually entered the enterohepatic but had not yet been exposed to hepatic CYP3A4 and thus reflect the separated intestinal CYP3A4 activity. D had a similar shape as the iv EBT curve, but a higher peak was reached. As can be seen in the C curve, intestinal CYP3A4 activity declined after 90–120 min. The median m value of 8.78% was significantly higher (+41%) than m of hepatic CYP3A4. The kinetics of intestinal CYP3A4 activity were characterized by a median tmax of 9 min and t1/2 of 21 min; the latter differing at a significant level from the hepatic curve.

After iv injection of 74 kBq of [N-methyl-14C]erythromycin (iv EUT; Fig. 1D), the percentage 14C recovery in urine showed a fast accumulation during the first 4 h [median value of C after 4 h (C4) was 10.82%], gradually reaching a plateau between 12 and 24 h after injection [median value of C after 24 h (C24) was 13.25%]. Mathematical analysis retained a t1/2 of 52 min; tmax could not be derived consistently (too few early urine collections possible to cover this very fast process). The total recovery at time infinite (m) was 13.26%, comparable with the measured value at 24 h. This corresponds to the fraction of undemethylated label that has not been excreted by hepatic/intestinal PGP in bile and feces but was eventually excreted in urine (intact or metabolized otherwise than by CYP3A4-mediated N-demethylation of the 14C-labeled methyl group). As a consequence, the m values of the EUT (iv and also po + deconvolved) curves are inversely correlated with hepatic and intestinal PGP activity.

Table 1. Results of iv and po EBT and EUT and deconvoluted curve in 32 healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Q1–Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EBT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D20</td>
<td>2.57</td>
<td>1.98–2.94</td>
</tr>
<tr>
<td>C4</td>
<td>5.12</td>
<td>4.14–5.69</td>
</tr>
<tr>
<td>m</td>
<td>6.24</td>
<td>5.58–7.46</td>
</tr>
<tr>
<td>t1/2</td>
<td>117</td>
<td>98–126</td>
</tr>
<tr>
<td>tmax</td>
<td>12</td>
<td>5–17</td>
</tr>
<tr>
<td>Dmax</td>
<td>2.04</td>
<td>1.73–2.68</td>
</tr>
<tr>
<td>po</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D60</td>
<td>0.23</td>
<td>0.14–0.29</td>
</tr>
<tr>
<td>C4</td>
<td>0.54</td>
<td>0.41–0.77</td>
</tr>
<tr>
<td>m</td>
<td>0.64</td>
<td>0.54–0.96</td>
</tr>
<tr>
<td>t1/2</td>
<td>114</td>
<td>95–140</td>
</tr>
<tr>
<td>tmax</td>
<td>58</td>
<td>42–76</td>
</tr>
<tr>
<td>Dmax</td>
<td>0.23</td>
<td>0.14–0.30</td>
</tr>
<tr>
<td><strong>Deconvolution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C24</td>
<td>13.25</td>
<td>11.82–15.30</td>
</tr>
<tr>
<td>m</td>
<td>13.26</td>
<td>11.95–15.27</td>
</tr>
<tr>
<td>t1/2</td>
<td>52</td>
<td>42–73</td>
</tr>
<tr>
<td>tmax</td>
<td>9</td>
<td>3–13</td>
</tr>
<tr>
<td>po</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C24</td>
<td>2.74</td>
<td>1.89–3.40</td>
</tr>
<tr>
<td>m</td>
<td>3.05</td>
<td>2.05–3.62</td>
</tr>
<tr>
<td>t1/2</td>
<td>503</td>
<td>407–621</td>
</tr>
<tr>
<td>tmax</td>
<td>167</td>
<td>79–288</td>
</tr>
<tr>
<td><strong>Deconvolution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td></td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>27.99</td>
<td>21.52–39.17</td>
</tr>
<tr>
<td>t1/2</td>
<td>268</td>
<td>207–340</td>
</tr>
<tr>
<td>tmax</td>
<td>123</td>
<td>89–136</td>
</tr>
</tbody>
</table>

Table 1. Results of iv and po EBT and EUT and deconvoluted curve in 32 healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Q1–Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EBT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D20</td>
<td>2.57</td>
<td>1.98–2.94</td>
</tr>
<tr>
<td>C4</td>
<td>5.12</td>
<td>4.14–5.69</td>
</tr>
<tr>
<td>m</td>
<td>6.24</td>
<td>5.58–7.46</td>
</tr>
<tr>
<td>t1/2</td>
<td>117</td>
<td>98–126</td>
</tr>
<tr>
<td>tmax</td>
<td>12</td>
<td>5–17</td>
</tr>
<tr>
<td>Dmax</td>
<td>2.04</td>
<td>1.73–2.68</td>
</tr>
<tr>
<td>po</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D60</td>
<td>0.23</td>
<td>0.14–0.29</td>
</tr>
<tr>
<td>C4</td>
<td>0.54</td>
<td>0.41–0.77</td>
</tr>
<tr>
<td>m</td>
<td>0.64</td>
<td>0.54–0.96</td>
</tr>
<tr>
<td>t1/2</td>
<td>114</td>
<td>95–140</td>
</tr>
<tr>
<td>tmax</td>
<td>58</td>
<td>42–76</td>
</tr>
<tr>
<td>Dmax</td>
<td>0.23</td>
<td>0.14–0.30</td>
</tr>
<tr>
<td><strong>Deconvolution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C24</td>
<td>13.25</td>
<td>11.82–15.30</td>
</tr>
<tr>
<td>m</td>
<td>13.26</td>
<td>11.95–15.27</td>
</tr>
<tr>
<td>t1/2</td>
<td>52</td>
<td>42–73</td>
</tr>
<tr>
<td>tmax</td>
<td>9</td>
<td>3–13</td>
</tr>
<tr>
<td>po</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C24</td>
<td>2.74</td>
<td>1.89–3.40</td>
</tr>
<tr>
<td>m</td>
<td>3.05</td>
<td>2.05–3.62</td>
</tr>
<tr>
<td>t1/2</td>
<td>503</td>
<td>407–621</td>
</tr>
<tr>
<td>tmax</td>
<td>167</td>
<td>79–288</td>
</tr>
<tr>
<td><strong>Deconvolution</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv</td>
<td></td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>27.99</td>
<td>21.52–39.17</td>
</tr>
<tr>
<td>t1/2</td>
<td>268</td>
<td>207–340</td>
</tr>
<tr>
<td>tmax</td>
<td>123</td>
<td>89–136</td>
</tr>
</tbody>
</table>

iv, Intravenous; po, oral; EBT, erythromycin breath test; EUT, erythromycin urine test; tmax, excretion peak time; t1/2, excretion half-life; m, total cumulative 14C recovery when time is infinite; Dmax, maximal excretion rate; D20/60, %dose/h recovery at 20/60 min; C4/24, cumulative %dose recovery at 4/24 h; Q1–Q3, interquartile range.

AJP-Gastrointest Liver Physiol • VOL 285 • SEPTEMBER 2003 • www.ajpgi.org
pared with iv administration (median C24, 2.74%). This resulted in a median $t_{1/2}$ of 503 min, $t_{\text{max}}$ of 167 min, and $m$ of 3%.

Figure 1F shows the median C curve after deconvolution of the po and iv EUT curves (Fig. 1, D and E). It represents the separated fraction of tracer that was absorbed along the intestinal tract and escaped excretion by the intestinal PGP system. As displayed, this process rose at a fast rate during the first 6–8 h, thereafter gradually tapering within 24 h. The calculation of kinetic parameters resulted in a $t_{1/2}$ of 268 min and $t_{\text{max}}$ of 123 min. The value of $m$ was 27.99%. This suggests that intestinal PGP activity was slower than CYP3A4 catabolism (see Fig. 1C) but longer lasting and quantitatively more important. It also demonstrates that around one fourth of orally administered tracer was absorbed systemically (Fig. 1F), but eventually only $\frac{1}{3}$% of the orally administered tracer escaped both (hepatic and intestinal) CYP3A4 metabolism plus excretion by hepatic and intestinal PGP (Fig. 1E).

No significant correlations were demonstrated between any of the iv and po measured and calculated parameters, neither for the EBT nor for the EUT.

Inter- and Intraindividual Variability

As expected, the between-subject (interindividual) variability was large for all measured and calculated parameters, with interquartile ranges for $m$ of ±25–30 (iv EBT/EUT) to ±65% (po EBT/EUT; Tables 1 and 2).

Repeated-measures ANOVA on three repeated po EBT and EUT results showed no significant within-subject variability for all measured parameters. The CV for three important parameters ($D_{\text{max}}$ and $m$ of the po EBT and $m$ of the po EUT) in eight subjects is shown in Table 2. The mean CV of all measured and fitted parameters ranged from 11 to 28%.

Fecal Excretion of $^{14}$C

The mean cumulative fecal excretion of $^{14}$C in five subjects after iv administration of 74 kBq of $[N$-methyl-$^{14}$C]erythromycin was 75% at day 5; the calculated $m$ was 82% (Fig. 2).

The mean cumulative fecal excretion of $^{14}$C in seven subjects after po administration of 74 kBq of $[N$-methyl-$^{14}$C]erythromycin was 87% at day 5; the calculated $m$ was 96%.

Influence of Gastric Acid Production and Gastric Emptying on the po EBT

Inhibition of gastric acid secretion by two different histamine II receptor blockers (cimetidine and raniti-
dine) caused no changes in the po EBT and EUT curves or the derived mathematical parameters compared with baseline in seven healthy volunteers.

Gastric emptying of the lactose-containing gelatin capsule, used in all po and iv EBT and EUT, was very fast in all 12 studied subjects: means ± SD gastric emptying coefficient (GEC) was 4.2 ± 0.3, \( t_{1/2} \) was 16 ± 9 min. In all subjects, no lag phase could be retained (\( t_{lag} \), 0).

Gastric emptying tests repeated in six subjects with 100 mg of \(^{13}\text{C} \) octanoic acid dissolved in water and in fruit juice gave identical results (water: GEC 4.0 ± 0.4, \( t_{1/2} \) 9 ± 6 min; fruit juice: GEC 4.1 ± 0.2, \( t_{1/2} \) 11 ± 9 min). Again, no lag phase could be retained.

**Healthy Volunteers: Baseline vs. Drug Induction (n = 12)**

The intake of itraconazole resulted in a significant flattening of the iv EBT curves (Fig. 3A). D20 decreased by 41%, \( m \) by 27%; \( t_{1/2} \) was prolonged by 24% and \( t_{max} \) by 17%. The shape of the po EBT curve (Fig. 3B) was even more altered: no peak at all was attained, and a descending slope was absent, making fitting of the curves and deconvolution impossible. D60 was decreased by 17%. The iv EUT (Fig. 4A) showed a significant increase in urinary \(^{14}\text{C} \) excretion (\( m \), +47%). \( t_{1/2} \) was significantly prolonged, but the overall shape of the curve was similar to baseline. The po EUT (Fig. 4B) showed even more pronounced changes, with \( m \) reaching seven times the baseline value. The kinetic parameter \( t_{max} \) did not change significantly from baseline, but \( t_{1/2} \) did shorten by 27% (Table 3). The separated intestinal EUT curves (Fig. 4C) slowed down after 8–10 h at a level of more than threefold the baseline value. No significant changes of the kinetic parameters were noted (Table 3). Because the EUT curves are inversely related to PGP activity, these data indicate that hepatic and, to a much larger extent, intestinal PGP activity was clearly diminished.

![Fig. 3. Median \(^{14}\text{C} \) recovery in breath in 12 healthy volunteers after iv (A) and po (B) administration of 74 kBq of \([^{14}\text{C}]\)erythromycin and derived deconvolution curves (C) in basal conditions (\( \bullet \)), after itraconazole (\( \square \)), rifampicin (\( \triangle \)), Seville orange juice (\( \triangleup \)), and grapefruit juice (\( \Diamond \)). Left: \(^{14}\text{C} \) recovery rate (\%/dose/h), right: cumulative \(^{14}\text{C} \) recovery (\%/dose).](http://ajpgi.physiology.org/)

*Fig. 3. Median \(^{14}\text{C} \) recovery in breath in 12 healthy volunteers after iv (A) and po (B) administration of 74 kBq of \([^{14}\text{C}]\)erythromycin and derived deconvolution curves (C) in basal conditions (\( \bullet \)), after itraconazole (\( \square \)), rifampicin (\( \triangle \)), Seville orange juice (\( \triangleup \)), and grapefruit juice (\( \Diamond \)). Left: \(^{14}\text{C} \) recovery rate (\%/dose/h), right: cumulative \(^{14}\text{C} \) recovery (\%/dose).*
erythromycin and derived deconvolution curves (volunteers after iv (B) and po (C)) administration of 74 kBq of $^{14}$C remained unaltered (Fig. 3m). Three- to fourfold increases in CYP3A4 activity curves (Fig. 3A) showed a doubling of $C_{\text{max}}$, $D_{60}$, $C_{4}$, and $t_{\text{max}}$ (Fig. 22%). The po and separated EUT curves were similar to the baseline curve (Fig. 4B).

For the iv EBT, the C and D curves of the transplant patients displayed significant alterations, comparable with those seen after induction with rifampicin, albeit less pronounced ($m_{1}$, 38%; Fig. 5, Tables 4 and 5). Considering kinetics, $t_{1/2}$ was similar, but $t_{\text{max}}$ came 3 min later. The po EBT curves showed a raise of 100% for $m$ and a slower $t_{1/2}$ and $t_{\text{max}}$. The separated intestinal CYP3A4 activity curves showed a 65% increase of $m$, however, with slower kinetics compared with healthy volunteers. Both po and iv EUT curves had lower activity levels ($m_{1}$, 44%). The iv EUT showed slower kinetics, and the po EUT showed faster kinetics. Because renal function was not normal in the patients, derivation of hepatic PGP activity from the iv EUT curves was not done. Due to the deconvolution procedure, the separated intestinal EUT curve is not influenced by renal function, which allows a clear interpretation. It showed a decreased ($m_{1}$, 18%) but faster activity, implicating raised intestinal PGP activity.

**DISCUSSION**

It is well known that there is a large interindividual variability of bioavailability of many orally administered drugs, such as immunosuppressive agents (e.g., calcineurin inhibitors). In this case, important variability can lead to over- and underimmunosuppression. Although hepatic and intestinal CYP3A4-mediated metabolism and excretion by PGP activity are considered to be very important in the elimination of drugs taken orally, to date, there is no method to measure the instantaneous activity of these pathways in humans. Major advances are being made in genotyping catabolic and excretory pathways eliminating xenobiotics, which

---

**Fig. 4.** Median cumulative $^{14}$C recovery in urine in 12 healthy volunteers after iv (A) and po (B) administration of 74 kBq of $^{14}$Cerythromycin and derived deconvolution curves (C) in basal conditions (●), after irtraconazole (■), rifampicin (●), Seville orange juice (▲), and grapefruit juice (●). For irtraconazole, the y-axis has been interrupted in B and C.

The intake of rifampicin resulted in a 50–87% increase in the iv EBT curves without significant changes in the kinetic parameters $t_{1/2}$ and $t_{\text{max}}$ (Fig. 3A). Three- to fourfold increases in $D_{\text{max}}$, $D_{60}$, $C_{4}$, and $m$ were noted in the po EBT curves; kinetic parameters remained unaltered (Fig. 3B). The separated intestinal CYP3A4 activity curves (Fig. 3C) showed a doubling of $m$ with similar kinetic properties as at baseline. The urinary excretion of tracer (iv EUT) dropped ±25% from baseline values with unchanged kinetics (Fig. 4A). The po EUT curves decreased ($m_{1}$, 44%) with significantly shorter $t_{1/2}$ (−33%) and $t_{\text{max}}$ (−74%; Fig. 4B). The separated intestinal EUT curves showed faster kinetics and a 21% decreased $m$ value (Fig. 4C). This corresponds to a increase of hepatic and intestinal PGP activity, the latter being more impressive.

Ingestion of Seville orange juice did not significantly change the iv curves (Figs. 3A and 4A). The po EBT curves however, were strongly diminished, with a 73% decrease of $D_{60}$, a 53% decrease of $D_{\text{max}}$ and $C_{4}$, and a 41% decrease of $m$. Kinetics were slowed significantly, with $t_{1/2}$ and $t_{\text{max}}$ prolonged with 42 and 88%, respectively (Fig. 3B). Consequently, the separated intestinal CYP3A4 curves (Fig. 3C) showed slower kinetics and activity ($m_{1}$, −22%). The po and separated EUT curves (Fig. 4B), and hence intestinal PGP activity (Fig. 4C), did not change significantly.

After intake of grapefruit juice, no significant changes of the iv EBT and EUT curves (Figs. 3A and 4A) were noted. The po EBT curves (Fig. 3B) changed in the same way as with Seville orange juice but not as drastic. The curves were flattened, with a 27% decrease of $m$ and slowed kinetics. As a consequence, the separated intestinal CYP3A4 curves (Fig. 3C) were changed in a similar way to those with Seville orange juice (slower kinetics; $m_{1}$, −11%). The po and separated EUT curves were similar to the baseline curve (Fig. 4B).
The iv-administered /H9262 g) does not inhibit these systems (1, 24, 51). The Table 3. Results of iv and po EBT and EUT and deconvolved curve in 12 healthy volunteers -methyl-13C]-erythromycin was reported by Paine et

ultimately may result in predicting maximal intestinal and hepatic elimination capacities of individual patients (2–3, 11, 19, 28). However, in the clinical setting with patients taking multiple drugs, a noninvasive, repeatable tool for in vivo phenotyping of the individual elimination capacity of orally ingested drugs may be useful to predict drug dosing and drug interactions.

We developed a 14C-labeled combined breath and urine test that allows evaluating both hepatic and intestinal CYP3A4 and PGP activity in humans. The test is based on the fact that erythromycin is a probe for both CYP3A4 and PGP and in the given dose (≥30 µg) does not inhibit these systems (1, 24, 51). The iv-administered [N-methyl-14C]erythromycin breath test is considered the gold standard for hepatic CYP3A4 activity (29, 43, 45, 51). Recently, a po EBT to quantify intestinal CYP3A4 activity using 500 mg of [N-methyl-14C]-erythromycin was reported by Paine et al. (39). Such a dose of erythromycin, however, can influence not only gastrointestinal motility but also CYP3A4 and PGP activity itself and therefore can have a major impact on test results, causing difficulties in estimating influences on CYP3A4 activity other than erythromycin induced (21, 23, 24, 51).

By combining iv with po administration of 74 kBq (30 µg) of [N-methyl-14C]erythromycin, by combining dynamic breath and urine collections, and by performing mathematical analysis of the measured curves, additional information could be obtained on intestinal CYP3A4 activity and hepatic plus intestinal PGP activity. After our data, we hypothesize that there is a different physiological behavior of the four processes described: the 1) hepatic and 2) intestinal CYP3A4 system and the 3) hepatic and 4) intestinal PGP system, with the PGP system exceeding the importance of the CYP3A4 system in the organism's defense against several xenobiotics.

Several observations support this hypothesis. First, ±80% of the tracer was collected in the feces by day 5 after iv injection. Because CYP3A4 catalysis of [N-methyl-14C]erythromycin ends in 14CO2 excreted in breath, ~80% of the tracer could not have been metabolized by hepatic nor intestinal CYP3A4. This, together with the low cumulative 14C recovery in the iv and po EBT, is a strong argument for the fact that the hepatic and intestinal PGP systems are probably quantitatively far more important than CYP3A4 as defense.

\*P < 0.05.
mechanisms against xenobiotics that are substrates of both systems. Whether $^{14}$C in feces was bound to intact or metabolized erythromycin (by pathways alternative to CYP3A4-mediated demethylation of the label) could not be determined, neither can it be stated that hepatic and intestinal excretory activity can be fully separated. Further validation using bile-derivation techniques could clarify this topic. Anyhow, the differences in kinetics between the iv vs. po EUT test results are at least compatible with a hepatic and an intestinal part of PGP activity.

Second, almost 100% of the tracer was collected after both po and iv administration (po: breath 0.6% + urine 3% + feces 96% = 99.6%; iv: breath 6% + urine 13% + feces 80% = 99%), and no significant correlations were demonstrated between any of the iv and po measured and calculated parameters. This suggests different ongoing processes (hepatic vs. intestinal).

Third, the early $^{14}$C peak and decline of activity after 4 h in the breath tests vs. only a gradual decline of $^{14}$C recovery after 12–24 h in urine collections is compatible with the known proximal main location of CYP3A4 (either liver or proximal intestinal mucosa) vs. the increasing concentration of PGP downward in the GI tract (maximal in distal ileum and colon).

Fourth, the alterations in po and iv EBT and EUT curves induced by different drugs were as expected by available literature data (Tables 3 and 5). The inhibitory effect of itraconazole and the inductive effect of rifampicin on both intestinal and hepatic CYP3A4 and PGP are clear and in accordance with reported literature. As expected, it was also demonstrated that itraconazole inhibited and rifampicin induced intestinal (CYP3A4 and PGP) more than hepatic systems in vivo (6, 15, 17, 18, 40, 43, 45). Seville orange juice, known to selectively inhibit intestinal CYP3A4 due to its compound, the flavanoid 6′-7′-bergamottin, also resulted in predictable changes of the EBT and unchanged EUT curves (9, 13, 35). Grapefruit juice showed only a decreased intestinal CYP3A4 activity, despite reports of
inhibition of intestinal CYP3A4 and PGP. However, the amount of grapefruit juice needed to obtain measurable effects (both in vitro and in vivo) is not well established and seems to vary from 250 ml to several glasses of either fresh or “double-strength reconstituted” lyophilized grapefruit juice (20, 22, 23, 27, 31, 33, 37, 41). Maybe the dose used in this study was too low. Moreover, grapefruit juice has recently been shown to inhibit organic anion transporter protein, a protein that is structurally related to PGP, but results in uptake of substances by the hepatocyte and probably the enterocyte (5, 8). In renal transplant recipients early after renal transplantation, induction of both the intestinal and hepatic CYP3A4 and PGP system was noted according to the inducing effects of corticosteroids (23, 29, 39). The use of a selective PGP inhibitor would probably have delivered a more stringent proof; however, these kinds of drugs are not available for human use to date.

Because erythromycin is known to undergo slow hydrolysis in an acid environment, the po EBT and EUT could be influenced by gastric acid production. Inhibition of gastric acid secretion with cimetidine and ranitidine showed no changes in the obtained curves and resulting parameters. This can be explained by several mechanisms: the gelatin capsule will probably provide at least some protection against an acid environment. Furthermore, the contact time with gastric secretions is very short when the capsule is ingested with a liquid after an overnight fast, as was confirmed by measurements of gastric emptying of the capsule.

Also, gastric emptying of water did not differ from that of fruit juice. This finding corroborates the thesis that changes provoked by the fruit juices reflect

### Table 4. Results of iv and po EBT and EUT and deconvolved curve in healthy volunteers and renal transplant patients

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Q1–Q3</th>
<th>Median</th>
<th>Q1–Q3</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CYP3A4</td>
<td></td>
<td>PGP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D20</td>
<td>2.57</td>
<td>1.98–2.94</td>
<td>3.18</td>
<td>2.33–4.33</td>
<td>0.005</td>
</tr>
<tr>
<td>C4</td>
<td>5.12</td>
<td>4.14–5.69</td>
<td>6.29</td>
<td>5.63–8.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>m</td>
<td>6.24</td>
<td>5.58–7.46</td>
<td>8.06</td>
<td>7.16–9.75</td>
<td>0.002</td>
</tr>
<tr>
<td>t1/2</td>
<td>117</td>
<td>98–126</td>
<td>105</td>
<td>97–111</td>
<td>0.113</td>
</tr>
<tr>
<td>tmax</td>
<td>12</td>
<td>5–17</td>
<td>15</td>
<td>9–23</td>
<td>0.068</td>
</tr>
<tr>
<td>Dmax</td>
<td>2.04</td>
<td>1.73–2.68</td>
<td>2.66</td>
<td>1.97–3.72</td>
<td>0.009</td>
</tr>
<tr>
<td>PO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D60a</td>
<td>0.23</td>
<td>0.14–0.29</td>
<td>0.34</td>
<td>0.19–0.42</td>
<td>0.009</td>
</tr>
<tr>
<td>C4a</td>
<td>0.54</td>
<td>0.41–0.77</td>
<td>0.82</td>
<td>0.78–1.23</td>
<td>0.001</td>
</tr>
<tr>
<td>m</td>
<td>0.64</td>
<td>0.54–0.96</td>
<td>1.26</td>
<td>0.99–1.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>t1/2</td>
<td>114</td>
<td>95–140</td>
<td>151</td>
<td>128–181</td>
<td>0.001</td>
</tr>
<tr>
<td>tmax</td>
<td>58</td>
<td>42–76</td>
<td>89</td>
<td>67–103</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dmax</td>
<td>0.23</td>
<td>0.14–0.30</td>
<td>0.30</td>
<td>0.26–0.43</td>
<td>0.003</td>
</tr>
<tr>
<td>Deconvolution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>8.78</td>
<td>6.61–14.03</td>
<td>14.42</td>
<td>10.22–22.75</td>
<td>0.009</td>
</tr>
<tr>
<td>t1/2</td>
<td>21</td>
<td>16–30</td>
<td>37</td>
<td>27–46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tmax</td>
<td>9</td>
<td>3–13</td>
<td>14</td>
<td>4–23</td>
<td>0.169</td>
</tr>
</tbody>
</table>

| EUT    |        |         |        |         |         |
| IV     |        |         |        |         |         |
| C24    | 13.25  | 11.82–15.30 | 7.48   | 6.64–9.92 | <0.001  |
| m      | 13.26  | 11.95–15.27 | 8.23   | 6.87–10.17 | <0.001  |
| t1/2   | 52     | 42–73    | 124    | 92–151   | <0.001  |
| tmax   | na     | na       | na     | na       | na      |
| PO     |        |         |        |         |         |
| C24    | 2.74   | 1.89–3.41 | 1.54   | 0.75–2.08 | <0.001  |
| m      | 3.00   | 2.05–3.62 | 1.67   | 0.82–2.19 | <0.001  |
| t1/2   | 503    | 407–621  | 392    | 293–472  | 0.017   |
| tmax   | 167    | 79–288   | 113    | 68–209   | 0.31    |
| Deconvolution | | | | | |
| m      | 27.99  | 21.52–39.17 | 22.95  | 12.00–33.42 | 0.034   |
| t1/2   | 268    | 207–340  | 174    | 88–258   | 0.015   |
| tmax   | 123    | 89–136   | 211    | 150–279  | 0.13    |

### Table 5. Summary of drug-induced influences on CYP3A4 and PGP activity expressed as relative change of m values in healthy volunteers at baseline conditions

<table>
<thead>
<tr>
<th>CYP3A4 Activity</th>
<th>PGP Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&lt;sub&gt;CYP3A4&lt;/sub&gt;</td>
<td>H&lt;sub&gt;PGP&lt;/sub&gt;</td>
</tr>
<tr>
<td>I&lt;sub&gt;CYP3A4&lt;/sub&gt;</td>
<td>I&lt;sub&gt;PGP&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug</th>
<th>CYP3A4 Activity</th>
<th>PGP Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Itraconazole</td>
<td>×0.75*</td>
<td>×0.68* ×0.27*</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>×1.8* ×2.14*</td>
<td>×1.32* ×1.27*</td>
</tr>
<tr>
<td>Seville orange juice</td>
<td>×0.99 ×0.78</td>
<td>×0.97 ×0.96</td>
</tr>
<tr>
<td>Grapefruit juice</td>
<td>×1.02 ×0.9</td>
<td>×0.94 ×1.04</td>
</tr>
<tr>
<td>Renal tx</td>
<td>×1.29* ×1.64*</td>
<td>×1.22*</td>
</tr>
</tbody>
</table>

CYP 3A4, cytochrome P-450; PGP, P-glycoprotein. *P < 0.05.
effects on the CYP3A4/PGP system and not on gastri
cic emptying.

The observation of a higher \( m \) value of the decon-
volved EBT (that reflects separated intestinal CYP3A4
activity) compared with that of the iv EBT suggests a
higher activity of intestinal than hepatic CYP3A4 in
vivo. This should be interpreted with caution however.
Because the administered \([N\text{-methyl-}^{14}\text{C}]\text{erythromycin}\) is labeled on only one of its two \(N\)-methyl groups, only one-half of the \( ^{14}\text{CO}_{2} \) generated by demethylation
will be measured as \( ^{14}\text{CO}_{2} \). This will cause an (maxi-
mally 50\%) underestimation of CYP3A4 activity by
both the iv and po breath test. Theoretically, this could
be corrected by doubling the \( m \) values. Due to the
mathematical properties of the deconvolution proce-
dure, this is not needed for the separated curve. The
fact that only one methyl group of the probe is labeled with
\( ^{14}\text{C} \) implies also that a portion of \( ^{14}\text{C} \) recovered in
urine originates from the CYP3A4 metabolized probe.
Theoretically, this portion could rise as high as the
percentage of \( ^{14}\text{CO}_{2} \) recovered in the breath test. The
contribution of this fraction to hepatic PGP activity as
measured by the iv EUT remains a matter of further
investigation. However, given the proportionality of the
\( ^{14}\text{C} \) recovery in breath (CYP3A4) vs. urine and
feces (PGP), this contribution to hepatic PGP activity
cannot exceed the fraction of CYP3A4 activity, under-
estimated by the breath test. As far as this effect on
measurement of intestinal PGP activity by the po EUT
is concerned, deconvolution of the iv and po EUT
curves results in its complete elimination. Thus the
deconvolved EUT curve only represents intestinal PGP
activity on the administered probe.

Statistical analysis of three baseline studies per-
formed by the same subjects showed that the test
results, albeit with a large interindividual variability,
are reproducible. Therefore repeated tests may be a
powerful instrument to determine whether side effects
or therapeutic windows are caused by in-
cidence of dis-
seases or therapeutics on CYP3A4 and PGP activity
rather than by day-to-day variability of a physiological
event.

Although further validation is needed, we developed
a combined labeled carbon breath and urine test that
allowed measuring the activity of both intestinal and
hepatic CYP3A4 and PGP activity with numerical cal-
culation of the kinetics of the different processes. The
test proved sufficiently stable day by day and suffi-
ciently sensitive to detect theoretically predicted phar-
macological modulations by different drugs in healthy
volunteers and after recent renal transplantation.
Also, radiation burden for the patients is minimal,
because the tracer is completely eliminated from the
body within 5 days. We conclude that the po and iv
erythromycin breath and urine test is a promising
noninvasive test to measure phenotypic intestinal and
hepatic CYP3A4 and PGP activity and may be a reli-
able tool for dose adjustment and prediction of many
immunosuppressive and other pharmacotherapeutics
in clinical practice. Further studies, however, are war-
ranted.

The ever-continuing support and enthusiasm of the clinical nurses
A. Herelikza, H. Wieland, R. Eerدهens, and G Melis and the
excellent technical support of S. Rutten, A. Luyppaerts, C. Dewit, and
R. Servaes and the other members of the Gastrointestinal Research
Center UZ Gasthuisberg are greatly acknowledged. The contribution
of the colleagues of the Department of Abdominal Transplant
Surgery (Prof. Dr. J. Pirenne and Prof. Dr. W. Coosemans) to successful
transplantations is much appreciated.

DISCLOSURES

B. D. Maes is holder of the Janssen-Cilag chair for nephrology at
the Katholieke Universiteit Leuven.

This work was supported by a Fujisawa grant, by the Fonds voor
Wetenschappelijk Onderzoek Grant A6691-G.0386.02, and by a grant
from the Stichting Christian Dubois.

REFERENCES

1. Achira M, Suzuki H, Ito K, and Sugiyama Y. Comparative
studies to dermine the selective inhibitors for p-glycoprotein
and cytochrome P450.

Population distribution and effects on drug metabolism of a
variant in the 5' promoter region of cyp 3a4. Clin Phar-

3. Brinkmann U, Roots I, and Eichelbaum M. Pharmacogenet-
ics of the human drug-transporter gene MDR1: impact of poly-
morphisms on pharmacotherapy. Drug Discov Today 15: 835–

4. Cakaloglu Y, Tredger JM, Devlin J, and Williams R. Im-
portance of cytochrome P-450IIIA activity in determining dosage
and blood levels of FK506 and cyclosporine in liver transplant

5. Cvetkovic M, Leake B, Fromm MF, Wilkinson GR, and Kim
RB. OATP and P-glycoprotein transporters mediate the cellular
uptake and excretion of fexofenadine. Drug Metab Dispos 27:

6. Damkier P, Hansen LL, and Broksen J. Rifaximin treatment
greatly increases the apparent oral clearance of quinidine.

7. De Wildt SN, Kearns GL, Leeder JS, and van den Anker
JN. Cytochrome P450 5A1 ontogeny and drug disposition. Clin

8. Dresser GK, Bailey DG, Leake BF, Schwarz UI, Dawson
PA, Freeman DJ, and Kim RB. Fruit juices inhibit organic
anion transporting polypeptide-mediated drug uptake to
decrease the oral availability of fexofenadine. Clin Pharmacol Ther

9. Edwards DJ, Fitzsimmons ME, Schuetz EG, Yasuda K,
Ducharme MP, Warbasse LH, Woster PM, Schuetz JD, and
Watkins PB. 6',7'-Dihydroxybergamottin in grapefruit juice
and Seville orange juice: effects on cyclosporine disposition,
enterocyte CYP3A4, and P-glycoprotein. Clin Pharmacol Ther 65:

10. Ghoos YF, Maes BD, Geypens BJ, Mys G, Hiele MI, Rut-
tgeers PJ, and Vantreppen G. Measurement of gastric empty-
ing rate of solids by means of by means of a carbon labelled

11. Guengerich FP. Pharmacogenomics of cytochrome P450 and
other enzymes involved in biotransformation of xenobiotics.

12. Guengerich FP. Characterisation of human cytochrome P450

13. Guo LQ, Fukuda K, Ohta T, and Yamazoe Y. Role of furano-
coumarin derivatives on grapefruit juice-mediated inhibition of

14. Hall SD, Thumel KE, Watkins PB, Lown KS, Benet LZ,
Paine MF, Mayo RR, Turgeon DK, Bailey DG, Fontana RD,


