IN VIVO EFFECT OF CLARITHROMYCIN ON MULTIPLE CYTOCHROME P450 S

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ABSTRACT:
The in vivo effects of oral clarithromycin administration on the in vivo activity of cytochrome P450 1A2, 2C9, and 2D6 were determined. The cytochrome P450 probes caffeine (CYP1A2), tolbutamide (CYP2C9), and dextromethorphan (CYP2D6) were administered as an oral cocktail prior to and 7 days after oral clarithromycin (500 mg twice daily) administration to 12 healthy male subjects. Blood and urine samples were collected and assayed for each of the compounds and their metabolites using high-performance liquid chromatography. The CYP1A2 indices, oral caffeine clearance (6.2 ± 3.3 l/h before and 5.7 ± 4.2 l/h after, p > 0.05) and the 6-h paraxanthine to caffeine serum concentration ratio (0.49 ± 0.3 before and 0.44 ± 0.3 after, p > 0.05), were unchanged following clarithromycin dosing. Neither the tolbutamide oral clearance (0.77 ± 0.28 l/h before and 0.72 ± 0.24 l/h after, p > 0.05) nor the tolbutamide urinary metabolic ratio (779 ± 294 before and 681 ± 416 after, p > 0.05) indices of CYP2C9 were altered by clarithromycin administration. In the case of CYP2D6, the dextromethorphan to dextrorphan urinary ratio was not significantly different before (0.021 ± 0.04) and after (0.024 ± 0.06) clarithromycin dosing. In conclusion, clarithromycin does not appear to alter the in vivo catalytic activity of CYP1A2, CYP2C9, and CYP2D6 in healthy individuals as assessed by caffeine, tolbutamide, and dextromethorphan, respectively.

Clarithromycin is a macrolide antibiotic, which is widely used for the treatment of a myriad of infections such as those caused by Hemophilus influenzae, Mycobacterium avium, and Helicobacter pylori. Clarithromycin is oxidatively metabolized to 14-(R)-hydroxyclarithromycin or N-demethylated to N-demethylclarithromycin and members of the CYP3A subfamily mediate these reactions (Rodrigues et al., 1997). Like erythromycin, clarithromycin is a potent mechanism-based inhibitor of CYP3A (Rodrigues et al., 1997). Clarithromycin reduces the clearance of a number of well characterized CYP3A substrates such as midazolam, cyclosporine, carbamazepine, and terfenadine (Albani et al., 1993; Honig et al., 1994; Jurima-Romet et al., 1994; Skekris et al., 1996; Gorski et al., 1998). For example, coadministration of clarithromycin with midazolam results in a 3-fold increase in midazolam AUC (Gorski et al., 1998). Likewise, the systemic clearance of midazolam is reduced by 50% and the elimination half-life is increased 3- to 4-fold (Gorski et al., 1998).

While the effects of clarithromycin on CYP3A activity have been well defined, effects on other CYP families have not been as well characterized. For example, the macrolide antibiotics erythromycin and clarithromycin impair theophylline metabolism (Weinerberger et al., 1977; Periti et al., 1992; Gillum et al., 1993; Abbott Laboratories, 2000). It is clear that theophylline is primarily metabolized by CYP1A2 (Robson et al., 1988; Ha et al., 1995). Although there is some evidence to suggest that the 8-hydroxylation of theophylline is partly catalyzed by CYP3A, it is considered a minor and clinically insignificant pathway of theophylline oxidation (Robson et al., 1988; Tjia et al., 1996). However, the concomitant administration of theophylline and clarithromycin (macrolide antibiotics) is cautioned against in the package insert (Abbott Laboratories, 2000).

Clarithromycin has also been shown to alter the metabolism of pimozide and clozapine, which are substrates of CYP1A2 and CYP3A (Pirmohamed et al., 1995; Linnet and Olesen, 1997; Desta et al., 1998; Flockhart et al., 2000). In addition, there is some evidence to suggest that CYP2D6 may contribute to the metabolism of clozapine (Fischer et al., 1992). The role of CYP2D6 in the disposition of pimozide is unclear. Desta et al. (1999) noted that the plasma concentrations of pimozide tended to be higher in CYP2D6 poor metabolizers compared with CYP2D6 extensive metabolizers but this difference was not significant. However, pimozide is a potent inhibitor of this enzyme (Desta et al., 1998). Additionally, there have been reports of an interaction between clarithromycin and the CYP2C9 substrate warfarin, resulting in enhanced anticoagulation (Recker and Kier, 1997; Oberg, 1998; Gooderham et al., 1999).

The interactions between clarithromycin and substrates of CYP1A2, CYP2C9, and CYP2D6 suggest that in addition to CYP3A, clarithromycin may have inhibitory effects on other P450 enzymes. Thus, it is unclear whether clarithromycin alters the catalytic activity of other human CYPs in vivo. If clarithromycin affected other human P450s in addition to CYP3A, there would be a risk of additional unidentified drug-drug interactions. The goal of this study is to determine the in vivo effects of clarithromycin on multiple CYPs by using a drug cocktail consisting of caffeine, tolbutamide, and dextromethorphan.
Materials and Methods

Chemicals. Acetaminophen, chlorpropamide, codeine, dextromethorphan, ethylmorphine, and \(\beta\)-glucuronidase were purchased from Sigma Chemical Co. (St. Louis, MO). Acetaminophen, methanol, isopropanol, chloroform, and tetrahydrofuran were purchased from Fisher Scientific (Pittsburgh, PA). 4-Hydroxytolbutamide and carboxytolbutamide were purchased from RBI/Sigma (Natick, MA) and GENTEST (Woburn, MA), respectively. Caffeine, paraxanthine, theobromine, theophylline, 8-hydroxycaffeine, dextromethorphan, 3-methoxyxanthin, and 3-hydroxymorphinan were purchased from RBI/Sigma. Other chemicals were of the highest grade commercially available.

Cocktail Validation Study Design. After Institutional Review Board approval, participants provided written consent to take part in the study. Fifteen (eight females and seven males) healthy, nonsmoking volunteers age 29 ± 6 years and weighing 75 ± 19 kg participated in the four-way randomized, crossover study. The four arms of the study were the oral administration of 1) caffeine (Vivarin, 200 mg; SmithKline Beecham, Pittsburgh, PA); 2) tolbutamide (500 mg, Orinase; Pharmacia and Upjohn, Bridgewater, NJ); 3) dextromethorphan HBr (30 mg, Robitussin; Whitehall-Robbins, Madison, NJ); and 4) caffeine, tolbutamide, and dextromethorphan simultaneously as a cocktail.

The four volunteers were determined to have an extensive metabolizer CYP2D6 phenotype using the dextromethorphan to dextrorphan urinary metabolic ratio. Volunteers with a dextromethorphan to dextrorphan urinary metabolic ratio greater than or equal to 0.3 (i.e., poor metabolizers) were excluded from participating in this study (Kupfer et al., 1984). 2) Blood samples were obtained through an indwelling catheter located in the volunteer's forearm at the following times: 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 6, 8, 12, 24, 48, 72, and 96 h post tolbutamide and cocktail administration. When caffeine and dextromethorphan were administered separately, blood samples were obtained as described for 24 h. Urine was collected in 12-h intervals for 96 h following tolbutamide and cocktail administration. When dextromethorphan or caffeine was administered separately, urine was collected in 12-h intervals for 72 and 24 h, respectively. A minimum of 5 days and a maximum of 10 days separated each arm of the study. Serum and urine were stored at −20°C until analysis.

Clarithromycin P450 Study Design. Twelve healthy, nonsmoking, nonethanol drinking, male volunteers age 31 ± 9 years weighing 85 ± 13 kg participated in the study. The clarithromycin interaction study was conducted in an identical manner to the cocktail validation study except for the following: 1) Prior to inclusion in the study, the CYP2D6 phenotype was determined using the dextromethorphan to dextrorphan urinary metabolic ratio. Volunteers with a dextromethorphan to dextrorphan urinary metabolic ratio greater than or equal to 0.3 (i.e., poor metabolizers) were excluded from participating in this study (Kupfer et al., 1984). 2) Blood samples were obtained through an indwelling catheter located in the volunteer’s forearm at the following times: 1, 2, 6, 12, 24, 48, 72, and 96 h after dosing.

A fixed order design was used because the duration of any effect of clarithromycin on CYPs is unknown. One week after initial cocktail dosing, the probe drug cocktail was administered, and blood and urine samples were collected as described above.

Caffeine and Paraxanthine. Serum caffeine and paraxanthine concentrations were determined using high-performance liquid chromatography with a modified version of a previously published method (Grant et al., 1983; Gorski et al., 2000). The assay was used to routinely measure caffeine and paraxanthine concentrations between 0.3 and 20 \(\mu\)g/ml. Duplicate quality control samples were evaluated with each batch of samples. Analytical results were considered acceptable if quality control samples were within ±15% of the nominal value. Interday precision values were 7, 3, and 6% for caffeine and 3, 2, and 2%, for paraxanthine concentrations of 0.7, 1.4, and 16 \(\mu\)g/ml, respectively. The accuracy values were 3, 1, and 1% for caffeine and 1, 2, and 1% for paraxanthine concentrations of 0.7, 1.4, and 16 \(\mu\)g/ml, respectively.

Tolbutamide and Metabolites. Tolbutamide, carboxytolbutamide, and 4-hydroxytolbutamide urine concentrations were determined using high-performance liquid chromatography using a modified version of a previously published method (Knodell et al., 1987; Gorski et al., 2000). Duplicate quality control samples were assessed with each batch of samples and considered acceptable if the determined value was within ±15% of the expected value. The interday precision for carboxytolbutamide and 4-hydroxytolbutamide at 2.8 \(\mu\)g/ml was 4.5 and 7%, respectively. The interday accuracy at 2.8 \(\mu\)g/ml for carboxytolbutamide and 4-hydroxytolbutamide was 1 and 1%, respectively. For tolbutamide concentrations of 0.1, 0.4, 1.0, and 4.0 \(\mu\)g/ml, the interday precision was 8, 10, 12, and 13%, respectively, and the interday accuracy was 1, 8, 7, and 8%, respectively.
Tolbutamide serum concentrations were determined using a modified version of a previously published method (Knodell et al., 1987). Briefly, 0.25 ml of a 40% phosphoric acid solution, 50 μl of a 0.1 mg/ml chlorpropanide (internal standard) solution, and 6 ml of chloroform were added to 0.25 ml of human serum. The samples were mixed vigorously for 30 min and the organic phase was transferred to a clean test tube, evaporated, and reconstituted with mobile phase (35% solution of acetonitrile in 50 mM potassium phosphate solution, pH 4.0). Tolbutamide and the internal standard were separated using a Beckman Ultrasphere column (5 μM × 4.6 mm i.d. × 250 cm; Torrance, CA) at a flow of 1.5 ml/min. Each batch of samples was processed with duplicate quality control values. Analytical results were considered acceptable if the estimated quality control value was within ±15% of the nominal value. The interday precision for tolbutamide at 0.28, 2.8, and 16 μg/ml was 10, 5, and 7%, respectively. The interday accuracy for tolbutamide at 0.14, 1.4, and 8 μg/ml was 2, 8, and 5%, respectively.

**Dextromethorphan and Metabolites.** Dextromethorphan and dextrophan were quantitated in human urine using a previously published method (Jones et al., 1996a,b). Duplicate quality control samples were processed with each batch of samples and used to determine acceptance of the analytical results as previously described (Jones et al., 1996b). All the interday coefficients of variation were less than 20%, except for 3-methoxymorphinan at a concentration of 2 ng/ml, which was 25%. The interday accuracies were less than 15% for all compounds.

**Pharmacokinetic Analysis.** The pharmacokinetics of tolbutamide and caffeine were determined using standard noncompartmental methods with the computer program WinNonlin (version 1.1; Pharsight, Mountain View, CA). The terminal elimination rate constant (k1), was calculated using the slope of the log-linear regression of the terminal elimination phase. Area under the plasma concentration versus time curve from zero to infinity (AUC0∞) was calculated using the log-linear trapezoidal rule up to the last measured time concentration (Clast) with extrapolation to infinity using Clast/k1. The elimination half-life was calculated as 0.693/k1. The clearance of tolbutamide, and caffeine was calculated as dose/AUC0∞.

In vivo activity of CYP1A2 was assessed using the oral clearance of caffeine. Others have demonstrated that the 6-h serum paraxanthine to caffeine concentration ratio correlated well with the oral clearance of caffeine (Jeppesen et al., 1996). Therefore, the 6-h paraxanthine to caffeine serum ratio was used as a CYP1A2 marker. The catalytic activity of CYP2C9 was determined using the oral clearance of tolbutamide. The 72-h cumulative urinary ratio of tolbutamide metabolites to tolbutamide [(4-hydroxytolbutamide + carboxytolbutamide)/tolbutamide] was also used as an index of CYP2C9 activity (Veronese et al., 1990). In vivo CYP2D6 activity was measured using the 24-h urinary metabolic ratio of dextromethorphan to dextrophan (Schmid et al., 1985).

**Statistical Analysis.** The difference between control and clarithromycin-treated group parameters was determined using a paired t test. The statistical analysis was performed using the computer program The SAS System for Windows (version 6.12; SAS Institute Inc., Cary, NC). A significance level of p ≤ 0.05 was used. Assuming 80% power and an α level of 0.05, a sample size of 12 was sufficient to observe a 20% change in caffeine clearance, 6-h serum paraxanthine to caffeine concentration ratio, and the oral clearance of tolbutamide. In the case of the urinary dextromethorphan to dextrophan metabolic ratio a sample size of 12 was sufficient to detect a 100% change.

**Results**

Fifteen (eight females, seven males; 14 extensive metabolizers, one poor metabolizer of CYP2D6) volunteers completed the cocktail validation portion of the study. The mean (±S.D.) caffeine serum concentrations are presented in Fig. 1A. No significant difference in the oral clearance of caffeine administered alone or as a component of the cocktail was observed (Fig. 1A Table 1). Likewise, the paraxanthine to caffeine serum concentration ratio was not significantly different (p > 0.05, n = 14) following the oral dosing of caffeine alone or together with dextromethorphan and tolbutamide.

The oral tolbutamide clearance (Fig. 1B; Table 1) was not significantly different following administration of tolbutamide alone and in combination with caffeine and dextromethorphan, respectively. One volunteer had a 5- to 6-fold lower tolbutamide oral clearance (0.15–0.21) compared with the other volunteers (0.96–1.03 l/h). Likewise, the elimination half-life was substantially greater (57 h) than the mean half-life determined for the other volunteers (8.4 ± 1.8 h). It appears that this individual is a poor metabolizer of CYP2C9, however this has not been confirmed by genotyping.

The dextromethorphan metabolic ratios for CYP2D6 following administration of dextromethorphan alone were compared with the metabolic ratios observed following dextromethorphan administration with caffeine and tolbutamide. No significant differences were observed between the urinary dextromethorphan to dextrophan ratio (Fig. 1C; Table 1). These findings indicate that caffeine, tolbutamide, and dextromethorphan can be administered as a cocktail.

The administration of clarithromycin twice daily for 1 week did not significantly alter the oral clearance of caffeine (Fig. 2C Table 2). Likewise, there was no significant difference between the area under caffeine concentration time curve before (38 ± 19 mg l/h) and after (41 ± 16 mg l/h) clarithromycin administration. CYP1A2 catalytic activity, as measured by the 6-h serum paraxanthine to caffeine concentration ratio and the ratio of the paraxanthine to caffeine area under concentration time curves, was not significantly (p > 0.05) altered by clarithromycin administration (Table 2). In good agreement with the work of others (Jeppesen et al., 1996), a strong correlation was observed between caffeine oral clearance and the 6-h serum paraxanthine to caffeine concentration ratio (n = 24, r = 0.94, p < 0.05; data not shown). Furthermore, a good correlation between caffeine oral clearance and the ratio of the paraxanthine to caffeine area under the concentration time curves (n = 24, r = 0.89, p < 0.05)

<table>
<thead>
<tr>
<th>CYP1A2</th>
<th>Alone</th>
<th>Cocktail</th>
</tr>
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<tbody>
<tr>
<td>Oral caffeine clearance (l/h) (n = 15)</td>
<td>5.0 ± 1.8</td>
<td>5.4 ± 2.8</td>
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<tr>
<td>Serum (6-h) paraxanthine/caffeine concentration ratio (n = 14)</td>
<td>0.42 ± 0.15</td>
<td>0.44 ± 0.19</td>
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<tr>
<td>CYP2C9</td>
<td></td>
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<tr>
<td>Extensive metabolizer (n = 14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral tolbutamide clearance (l/h)</td>
<td>0.96 ± 0.26</td>
<td>1.03 ± 0.25</td>
</tr>
<tr>
<td>Urinary tolbutamide metabolic ratio</td>
<td>630 ± 215</td>
<td>678 ± 238</td>
</tr>
<tr>
<td>Poor metabolizer (n = 1)</td>
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<tr>
<td>Oral tolbutamide clearance (l/h)</td>
<td>0.15</td>
<td>0.21</td>
</tr>
<tr>
<td>Urinary tolbutamide metabolic ratio</td>
<td>45</td>
<td>96</td>
</tr>
<tr>
<td>CYP2D6</td>
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<td></td>
</tr>
<tr>
<td>Extensive metabolizer (n = 14)</td>
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<tr>
<td>Urinary dextromethorphan/dextrorphan metabolic ratio</td>
<td>0.066 ± 0.089</td>
<td>0.056 ± 0.063</td>
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<tr>
<td>Poor metabolizer (n = 1)</td>
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<tr>
<td>Urinary dextromethorphan/dextrorphan metabolic ratio</td>
<td>3.2</td>
<td>3.4</td>
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</table>
was observed (Fig. 3A). Likewise, a correlation between the 6-h paraxanthine/caffeine ratio and paraxanthine AUC/caffeine AUC ratio was observed (data not shown; \( n = 24, r = 0.93, p < 0.05 \)).

The in vivo activity of CYP2C9 was assessed by the urinary tolbutamide metabolic ratio and the oral clearance of tolbutamide (Fig. 2; Table 2). The urinary metabolic ratio decreased 13% from 860 ± 330 to 750 ± 460 but this change was not significant (\( p > 0.05 \), Table 2). The oral clearance of tolbutamide was not significantly different before (0.77 ± 0.28 l/h) and after (0.72 ± 0.24 l/h) clarithromycin administration. Surprisingly, the oral clearance of tolbutamide was not highly correlated with the tolbutamide urinary ratio (Fig. 3B). CYP2D6 activity was determined using the 24-h dextromethorphan/dextrorphan urinary ratio, which was also unchanged after clarithromycin administration, 0.019 ± 0.039 before and 0.027 ± 0.063 after (\( p = 0.29 \)) (Fig. 2; Table 2).

Discussion

The current study was designed to evaluate whether clarithromycin altered the in vivo activity of CYP1A2, CYP2C9, and CYP2D6 in addition to its established effects on CYP3A4. These four enzymes are the principle drug-metabolizing enzymes in humans and account for the metabolism of >90% of administered drugs that require biotransformation for elimination. Each of the probe drugs used, tolbutamide, caffeine, and dextromethorphan, have been validated individually as effective and specific probes for CYP1A2, CYP2C9, and CYP2D6, respectively.

It is clear that macrolide antibiotics such as troleandomycin, erythromycin, and clarithromycin are potent irreversible inhibitors of CYP3A-mediated biotransformations both in vitro and in vivo. We have previously demonstrated that clarithromycin significantly inhibits CYP3A activity in vivo (Gorski et al., 1998). In that study, we used intravenous midazolam, and an oral solution of the stable isotope \(^{15}\)N\(_3\)-midazolam was administered simultaneously both before and after 7 days of clarithromycin to 16 healthy individuals. Systemic clearance decreased significantly from 29 to 10 l/h, suggesting an inhibition of hepatic CYP3A by clarithromycin (Gorski et al., 1998). However, oral bioavailability increased 2.5-fold after clarithromycin administration (\( F = 0.31 \pm 0.1 \) before treatment, \( F = 0.75 \pm 0.02 \) after treatment) (Gorski et al., 1998). This increase in bioavailability could not be accounted for entirely by effects on hepatic metabolism and indicates that clarithromycin also inhibits gut wall metabolism. Thus, from these human data, 7 days of clarithromycin has substantially reduced CYP3A activity in the gastrointestinal epithelium and liver.

It is well established that CYP1A2 plays a prominent role in the \( N \)-demethylations of theophylline and that the 8-hydroxylation of theophylline is catalyzed by both CYP2E1 and CYP1A2 (Robson et al., 1988). In agreement with the in vitro data, the clearance of theophylline is increased by concurrent smoking and decreased following coadministration of known CYP1A2 inhibitors such as fluvoxamine and ciprofloxacin (Rasmussen et al., 1997). However, it is also clear that other agents such as the macrolide antibiotics, known inhibitors of CYP3A, and rifampin, a known CYP3A inducer, alter the clearance of theophylline in vivo (Adebayo et al., 1989; Upton, 1991a,b; Periti et al., 1992). For instance, coadministration of theophylline with troleandomycin results in a 50% reduction in the systemic clearance of theophylline and a corresponding 2-fold increase in the AUC (Weinberger et al., 1977). However, the ability of erythromycin to alter the disposition of theophylline is not as clear with numerous reports supporting and refuting an interaction between theophylline and erythromycin (Upton, 1991a,b). Others have indicated that clarithromycin impairs the metabolism of theophylline and enhances the anticoagulant properties of warfarin in vivo (Recker and Kier, 1997; Oberg, 1998; Gooderham et al., 1999; Abbott Laboratories, 2000). This raises the question as to whether clarithromycin and macrolide antibiotics in general might have broader inhibitory capabilities in humans. Due to the potential for theophylline toxicity, we examined the potential for clarithromycin to alter the in vivo CYP1A2 activity using the well characterized CYP1A2 probe caffeine.

In contrast to the observations of others, clarithromycin failed to alter the in vivo CYP1A2 activity as assessed by the disposition of caffeine. Similarly, Rasmussen et al. (1997) reported a poor correlation between the 6-h paraxanthine to caffeine plasma ratio and caffeine urinary metabolic ratio and the oral clearance of theophylline and the partial theophylline metabolite clearances in vivo. Taken
disposition of theophylline. Alternatively, troleandomycin and clarithromycin may inhibit the CYP2E1-mediated metabolism of theophylline, but there is little data to support this line of reasoning.

The use of the cocktail of probes allowed data to be collected for three separate enzyme systems, simultaneously. In addition to limiting intraindividual differences, a cocktail of probes also enables one to study a large number of enzymes in a shorter duration of time. Frye et al. (1997) administered a five-drug cocktail, the “Pittsburgh cocktail,” verifying that multiple probe drugs could be administered together without significant metabolic interactions. However, the utility of the Pittsburgh cocktail is limited because 1) one of the probes used, debrisoquine, is not available for use in the United States; 2) there is no CYP2C9 probe, which is a major drug-metabolizing P450 enzyme; 3) dapsone is used as an index of CYP3A activity; and 4) a limitation of the Pittsburgh cocktail approach is that urinary ratios for caffeine are used as a marker for CYP1A2. Typically, urinary ratios do not correlate with serum AUC for caffeine. Our approach uses three readily available and generally inert drugs, namely, caffeine, tolbutamide, and dextromethorphan and all are simple to administer and have been previously validated as probes individually.

In conclusion, we have verified that there is no interaction between caffeine, tolbutamide, and dextromethorphan when coadministered. Additionally, a good correlation was observed between the 6-h serum paraxanthine to caffeine ratio and the serum caffeine AUC, oral caffeine clearance, and the paraxanthine to caffeine area under the concentration time curve ratio, indicating that using a single time point to measure enzyme activity simplifies and could potentially shorten the duration of drug interaction and metabolism studies while limiting the amount of blood obtained from the individual. Finally, we found that clarithromycin had no significant effect on the catalytic activities of CYP1A2, CYP2C9, and CYP2D6 as assessed by caffeine, tolbutamide, and dextromethorphan and all are simple to administer and readily available and generally inert drugs, namely, caffeine, tolbutamide, and dextromethorphan, respectively.

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References


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