In recent years there has been a dramatic increase in both the availability and the use of various nutraceutical preparations in the United States. Over-the-counter accessibility, aggressive marketing, and the general consumer misconception that “natural” herbal preparations are better and safer than synthetic prescription medications are combining to make St John’s Wort one of the most popular herbal products currently on the market.1-3

Herbals are classified as “dietary supplements” or “food products” by the US Food and Drug Administration and are not subject to the same stringent regulations imposed on other over-the-counter and prescription medications. As a result there is a lack of critical information regarding the pharmacology, toxicology, pharmacokinetics, safety, and drug interactions of these compounds. Regardless of efficacy, without information about the potential for drug interactions, it is impossible to make responsible decisions about the treatment of patients using St John’s Wort.

Consumers purchasing St John’s Wort typically have concomitant disease(s), take other medications, or both: the populations most likely to have adverse drug events.2-7 They are attracted to this product by its broad

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**PHARMACOKINETICS AND DRUG DISPOSITION**

**St John’s Wort: Effect on CYP3A4 activity**

**Background:** St John’s Wort is a widely used herbal product. Information regarding its potential for drug interactions is required for responsible treatment of patients using St John’s Wort. CYP3A4 is a metabolic enzyme implicated in most clinically significant drug-drug interactions.

**Objective:** To determine the in vivo effect of reagent-grade St John’s Wort extract on CYP3A4 activity through evaluation of urinary 6β-hydroxycortisol/cortisol ratios.

**Methods** Thirteen subjects ranging in age from 18 to 25 years participated in this unblinded, multiple-dose, single-treatment before-after trial conducted in a university-based pharmacokinetics and biopharmaceutics laboratory. Each subject ingested a 300-mg tablet of reagent-grade St John’s Wort extract standardized to 0.3% hypericin three times a day for 14 days. Baseline and posttreatment CYP3A4 activity was assessed with the urinary 6β-hydroxycortisol/cortisol ratio after a 24-hour urine collection.

**Results** The mean ± SD urinary 6β-hydroxycortisol/cortisol ratio significantly increased (P = .003) from a baseline value of 7.1 ± 4.5 to 13 ± 4.9. The mean ± SD percentage increase was 114% ± 95%, with a range from -25% to 259%. All but one subject had an increase in the ratio.

**Conclusions** Treatment with St John’s Wort for 14 days resulted in significant increases in the urinary 6β-hydroxycortisol/cortisol ratio. This finding suggests that St John’s Wort is an inducer of CYP3A4.

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Carol A. Roby, PharmD, MS, Gail D. Anderson, PhD, Eric Kantor, BA, Donna A. Dryer, MD, and Aaron H. Burstein, PharmD
Baltimore and Bethesda, Md, and Seattle, Wash

In recent years there has been a dramatic increase in both the availability and the use of various nutraceutical preparations in the United States. Over-the-counter accessibility, aggressive marketing, and the general consumer misconception that “natural” herbal preparations are better and safer than synthetic prescription medications are combining to make St John’s Wort one of the most popular herbal products currently on the market.1-3

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Consumers purchasing St John’s Wort typically have concomitant disease(s), take other medications, or both: the populations most likely to have adverse drug events.2-7 They are attracted to this product by its broad
To normalize for circadian variations has been shown to be a nonspecific marker of CYP3A4 activity. Surrogate markers are widely used to measure and compare the activity of CYP450 microsomal enzymes in vivo and in vitro. To normalize for circadian variations in 6-β-hydroxycortisol production, the ratio of urinary 6-β-hydroxycortisol to cortisol is determined and used to evaluate CYP3A4 activity. To determine this study was to determine the in vivo effect of reagent-grade St John’s Wort extract on CYP3A4 activity through evaluation of the urinary 6-β-hydroxycortisol/cortisol ratio.

METHODS

This study was approved by the University of Maryland Institutional Review Board (Baltimore, Md). All participants provided written informed consent. The study was conducted at the School of Pharmacy Pharmacokinetics and Biopharmaceutics Laboratory, University of Maryland, Baltimore.

Chemicals and reagents. St John’s Wort (300-mg reagent-grade tablets, lot 180632) was purchased from Hypericum Buyers Club (Los Angeles, Calif). The St John’s Wort preparation used in this study was produced with the alcohol extraction procedure described in the German Commission E monograph for St John’s Wort, standardized to 0.3% hypericin (confirmed in our laboratory by a United States Pharmacopeia/National Formulary method). All chemicals used in the analysis of urine samples were reagent or analytical grade.

Volunteers and study design. Thirteen healthy volunteers (four men and nine women) from 18 to 45 years old were enrolled in and completed this unblinded multiple-dose protocol. The target sample number (n) was determined on the basis of a priori selection of α = .05, β = .2, and σ = .4, and a minimal detectable CYP activity difference before and after treatment of 50%.

The protocol was divided into two phases. Phase 1 consisted of screening and urine collection to determine baseline CYP3A4 activity. Phase 2 involved administration of St John’s Wort and urine collection to determine posttreatment CYP3A4 activity. Participants were considered to be starting the protocol on day 1 of phase 1, and all study restrictions were continuously applied until phase 2 was completed.

During screening, a blood sample was drawn for complete blood cell count and Sequential Multiple Analysis-18. Eligible participants were nonsmokers, took no other medication (including hormonal birth control), and were willing to abstain from alcohol and caffeine for 2 weeks before and during the protocol. In addition, subjects were requested to abstain from grapefruit, grapefruit juice, herbal dietary supplements, and herbal tea during the study. Female participants had to show that they were not currently pregnant (urine β-human chorionic gonadotropin) and agree to use medically approved nonhormonal birth control during the study and for one menstrual cycle beyond the duration of the protocol. Volunteers were ineligible if they had used any medications, including over-the-counter or prescription products, within 2 weeks of the study start date; had an estimated creatinine clearance of <80 mL/min (Cockcroft-Gault); had a medical history significant for kidney disease, liver disease, coronary artery disease, congestive heart failure, cerebrovascular disease, or peripheral vascular disease; had an abnormal complete blood cell count; had liver function tests greater than twice the upper limit of normal; had body weight more than 20% above or below the ideal body weight range as defined by the Metropolitan Life height, weight, and body tables; or had known sensitivity to St John’s Wort or hypericin or any other constituents of products used in this study.

Phase 1: Baseline CYP3A4 activity determination. All volunteers collected all urine for a specified 24-hour period. Urine was stored at room temperature during collection and until processing. The total 24-hour volume was recorded, and aliquots were frozen and stored at –20°C before analysis. Aliquots of the phase-1 24-hour urine were used to establish baseline (pretreat-
ment) CYP3A4 isozyme activity by evaluation of the urinary 6-ß-hydroxycortisol/cortisol ratio.13,22

Phase 2: Treatment and posttreatment CYP3A4 activity determination. Participants received a 15-day supply of 300-mg reagent-grade St John’s Wort tablets. Subjects were instructed to take one 300-mg tablet three times per day by mouth for 14 days. On day 15 of the treatment protocol, participants collected all urine for 24 hours. Urine samples were stored at room temperature during collection and until processing. St John’s Wort treatment was continued during the 24-hour urine collection period. Total urine volumes were measured and recorded, and aliquots were frozen and stored at –20°C before analysis was done.

Analytic methods. Urine samples for determination of 6-ß-hydroxycortisol and cortisol concentrations were analyzed with a modification13 of a previously described validated HPLC method.22 Samples were analyzed in duplicate during the same assay run. The sensitivity of the assay was 5 ng/mL for both 6-ß-hydroxycortisol and cortisol. Intraday and interday coefficient of variation values for both 6-ß-hydroxycortisol and cortisol were <5% and <10%, respectively.

To confirm the specificity of the HPLC assay for 6-ß-hydroxycortisol and cortisol, urine samples from the control and treatment phases of six of the subjects were reanalyzed with liquid chromatography–mass spectrometry. Liquid chromatography–mass spectrometry was performed with a Hewlett-Packard model HP1100 liquid chromatography system (Hewlett-Packard Co, Palo Alto, Calif) equipped with both a diode array and a mass spectrometer detector. Peak identity values for each analyte and internal standard were confirmed with selective ion monitoring at m/z = 363.2 for cortisol, m/z = 379.2 for 6-ß-hydroxycortisol, m/z = 377.2 for 6-ß-hydroxy cortisol, and m/z = 251.1 for heptabarbital (INN, heptabarb). Retention times from selective ion monitoring were comparable to those generated by ultraviolet detection. Ratios of 6-ß-hydroxycortisol and cortisol were compared in the subjects in control and treatment phases with the data obtained by HPLC.

Statistics. Comparison of urinary 6-ß-hydroxycortisol/cortisol ratios after treatment with values at baseline was by the two-tailed paired Student t test, with statistical significance declared at P < .05.

RESULTS

All enrolled subjects completed the study protocol. Compliance was assessed by subject reporting of missed doses at the end of phase 2. All subjects reported that they had taken all dispensed study medication as directed. St John’s Wort was well tolerated with no reported adverse events.

Nine female and four male patients were enrolled in this study. The mean ± SD age was 30 ± 7.5 years, with a range of 20 to 41 years. All women in the study were premenopausal. None had undergone hysterectomy or oophorectomy.

Results of urinary 6-ß-hydroxycortisol/cortisol ratio analysis at baseline and after 14 days of treatment with St John’s Wort are reported in Table I and displayed in Fig 1. All except one subject (subject 6) showed an increased urine ratio after treatment. Subject 6 had a 25% posttreatment decrease from the baseline urine ratio. At

### Table I. Urine 6-ß-hydroxycortisol/cortisol ratios at baseline and after administration of St John’s Wort for 14 days

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Baseline 6-ß-hydroxycortisol/cortisol urine ratio</th>
<th>Posttreatment 6-ß-hydroxycortisol/cortisol urine ratio</th>
<th>Change from baseline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.0</td>
<td>16.7</td>
<td>109</td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>13.7</td>
<td>259</td>
</tr>
<tr>
<td>3</td>
<td>9.5</td>
<td>11.3</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>6.3</td>
<td>20.3</td>
<td>222</td>
</tr>
<tr>
<td>5</td>
<td>8.8</td>
<td>12.9</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>20.8</td>
<td>15.5</td>
<td>-25</td>
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<tr>
<td>7</td>
<td>5.0</td>
<td>5.7</td>
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<td>8</td>
<td>4.8</td>
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<td>54</td>
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<tr>
<td>9</td>
<td>4.4</td>
<td>14.2</td>
<td>222</td>
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<td>10</td>
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<td>9.2</td>
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<td>11</td>
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<td>20.9</td>
<td>243</td>
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<tr>
<td>12</td>
<td>6.5</td>
<td>14.9</td>
<td>129</td>
</tr>
<tr>
<td>13</td>
<td>3.2</td>
<td>6.4</td>
<td>100</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>7.1 ± 4.5</td>
<td>13 ± 4.9*</td>
<td>114 ± 95</td>
</tr>
</tbody>
</table>

*P = .003 compared with baseline value.
baseline the mean ± SD urinary 6-β-hydroxycortisol/cortisol ratio was 7.1 ± 4.5. After treatment the mean ± SD ratio significantly increased (P = .003) to 13 ± 4.9. The mean ± SD percent increase of posttreatment urinary 6-β-hydroxycortisol/cortisol ratios from baseline was 114% ± 95%, with a range from –25% to 259%.

**DISCUSSION**

In our study, reagent-grade St John’s Wort taken for 14 days at the dose recommended for the treatment of mild to moderate depression (one 300-mg 0.3% hypericin standardized tablet orally three times per day)

Fig 1. Urinary 6-β-hydroxycortisol/cortisol ratios (y-axis) at baseline and after 14 days of St John’s Wort (treatment) for individual subjects (open circles). Heavy line (solid circles) represents the mean ratio at baseline and after 14 days of treatment.

was associated with a significant increase in the mean urinary 6-β-hydroxycortisol/cortisol ratio. This result suggests that St John’s Wort taken at recommended doses induces CYP3A4 activity.

Subjects received St John’s Wort for 14 days before CYP3A4 activity was reassessed. This duration of treatment was chosen as an interval that allowed participants to be exposed to St John’s Wort for approximately 1 week at assumed steady-state concentrations based on the maximum reported hypericin half-life of 40 hours. The duration of treatment may influence the ability to detect statistically significant induction of CYP3A activity. Ereshefsky et al. reported a lack of interaction of St John’s Wort with CYP3A4 after participants had been treated for 8 days with 300 mg of 0.3% hypericin standardized reagent-grade St John’s Wort three times daily. In that study, CYP3A4 activity was determined as the urine dextromethorphan/3-methoxymorphinan ratio. The investigators described a nonsignificant decrease in the mean ± SD ratio from 11.2 ± 10.2 at baseline to 6.1 ± 3.2 after 8 days of treatment, suggestive of increased CYP3A4 activity.

Similar inability to detect a statistically significant effect of St John’s Wort on CYP3A4 activity was found in a study that used alprazolam as a marker for CYP3A4 activity. After a 0.3% hypericin standardized St John’s Wort formulation was administered three times daily for 4 days, no changes were observed compared with baseline in time to maximal concentration, maximal concentration, terminal elimination half-life, and area under the concentration–time curve (AUC) extrapolated to infinity. There were a number of limitations to that study. Two doses of alprazolam were used, with three subjects receiving 1-mg doses and four subjects receiv-
ing 2-mg doses. On inspection of the individual subject data, it appears that the lack of a change in AUC may have been in part attributable to the small dose and limited duration of detectable concentrations (12 hours), thereby precluding reliable estimation of alprazolam terminal elimination rate constants and extrapolation of AUC to infinity. Inspection of the data for subjects receiving the 2-mg dose reveals a clear trend toward a decreased AUC after treatment with St John’s Wort. The small number of subjects studied and the short duration of St John’s Wort treatment add to the difficulty in detecting a significant effect. Although the choice of marker and its variability in the previously described studies may represent one explanation for the inability to detect a significant difference, an alternative explanation may be that 4 and 8 days of treatment is insufficient to elicit the full magnitude of the induction. Current research in our laboratory is evaluating the time course of induction.

All but one subject (subject 6) showed an increase in urinary 6-β-hydroxycortisol/cortisol ratio after 14 days of treatment. Induction at baseline caused by an unreported protocol violation can be hypothesized but not proved. A review of this subject’s records provided no plausible explanation, such as acute illness or protocol violation for the result. In our study CYP3A4 genotypes were not determined. Therefore it is unknown whether the phenotypic variance of subject 6 had a genotypic explanation. It is plausible that subject 6 may be representative of a group of patients at the upper region of a highly variable distribution of CYP3A4 activity. Recently a CYP3A4 genotype variant was reported. However, no corresponding phenotypic change was noted in black American individuals who possessed the homozygous CYP3A4 5’ promoter region A→G point mutation. It is uncertain whether the lack of corresponding CYP3A4 phenotypic change in the presence of a genotype variant would be applicable to other races or ethnic backgrounds because racial or ethnic differences in genotype-phenotype relationships have been described for CYP2D6.

The results of our study are consistent with findings published in abstract form by Kerb et al. In that study 50 healthy subjects received 2 weeks of treatment with 300-mg hypericum administered three times daily. Urinary excretion rates of 6-β-hydroxycortisol increased from mean ± SD pretreatment values of 257.7 ± 126.2 µg/d to 363.2 ± 175.5 µg/d (P = .000011). Urinary cortisol excretion did not differ between pretreatment (35.0 ± 27.2 µg/d) and posttreatment (30.3 ± 18.5 µg/d) evaluations.

The urinary 6-β-hydroxycortisol/cortisol ratio has been shown to be a reliable in vivo surrogate marker of CYP3A4 enzyme activity. Cortisol is metabolized primarily by CYP3A4 and, to a lesser extent, by renal CYP3A5 to 6-β-hydroxycortisol with excretion by the kidneys. Changes in the urinary excretion of this noninvasive marker substance have shown correlation with CYP3A4 isozyme induction and inhibition. Diurnal fluctuations in endogenous cortisol production and, secondarily, the urinary excretion of 6-β-hydroxycortisol have been documented. The 6-β-hydroxycortisol/cortisol ratio has been shown to be constant over a 24-hour period, and therefore its use is preferred over urinary 6-β-hydroxycortisol excretion for assessment of CYP3A4 activity. Because cortisol and 6-β-hydroxycortisol are endogenous substances, there is no risk to the subject when they are used to monitor microsomal enzyme activity. Although the renal tubules have CYP3A5 activity capable of metabolizing cortisol to 6-β-hydroxycortisol, the effects observed in this study are likely related to induction of CYP3A4 because CYP3A5 has not been shown to be inducible and is generally present in smaller concentrations.

The clinical significance of the effect observed in our study is uncertain. Comparison of our results with previously published studies that evaluated known clinically important inducers of CYP3A4 activity may allow for hypotheses about the anticipated magnitude of effect. Rifampin (INN, rifampicin) has been shown to elicit an approximate threefold increase in the urinary 6-β-hydroxycortisol/cortisol ratio. Treatment with 600 mg rifampin daily for 14 days resulted in a 320% increase in the morning spot 6-β-hydroxycortisol/cortisol ratio and a 137% increase in the ratio after a 24-hour urine collection. Our results show an approximate doubling in the posttreatment urinary 6-β-hydroxycortisol/cortisol ratio. This comparison of our findings with the effects of a known inducer of the urinary 6-β-hydroxycortisol/cortisol ratio suggests that treatment with St John’s Wort at the recommended dose may result in a clinically significant induction of CYP3A4. The magnitude of the interaction on specific substrates cannot be reliably predicted and should be determined in specific clinical investigations.

Since this study was completed, a clinical study and case reports have substantiated our findings. It has also recently been reported that administration of St John’s Wort for 2 weeks reduced the AUC of indinavir by a mean of 57% ± 19% and decreased the extrapolated 8-hour indinavir trough level by 81% ± 16%. Case reports describe decreased cyclosporine and oral contraceptive concentrations in patients taking St John’s Wort. Additional reports suggest effects on other CYP isoforms.
and P-glycoprotein. A case report that described decreased theophylline concentrations and an associated in vitro study suggests that St John’s Wort is an inducer of CYP1A2. Clinical studies have shown that St John’s Wort reduces digoxin exposure and trough concentrations through presumed induction of P-glycoprotein and reduces phenprocoumon concentrations. A more complete characterization of the effect of St John’s Wort on CYP450 isozyme activity, P-glycoprotein, and the time course for its induction of CYP3A4 is warranted.

This study shows that there may be clinically significant drug-drug interactions between 0.3% hypericin standardized reagent-grade St John’s Wort extract and substances metabolized through the CYP3A4 isozyme. Specifically, reductions in therapeutic efficacy at standard doses of important CYP3A4 substrates, including oral contraceptives, certain antiretrovirals, antiepileptics, calcium channel blockers, cyclosporine, fentanyl, and select chemotherapeutics, antibiotics, and antifungals, may be observed. The magnitude of these potential interactions cannot be extrapolated from the results of this study. Therefore it is imperative that agent-specific protocols be undertaken to evaluate these interactions.

Because of a lack of regulation, there is much variability in production methods and product content of St John’s Wort preparations sold in the United States. This lack of consistency means that our results may not be equally extrapolated to all available (ie, nonreagent grade) St John’s Wort products currently on the market. Until substrate-specific information is available, careful consideration and monitoring are warranted when St John’s Wort is combined with medications known to be metabolized by CYP3A4.

References

23. Kerb R, Brockemoller J, Staffeldt B, Ploch M, Roots I. Single-dose and steady-state pharmacokinetics of hyper-


