St. John's Wort Does Not Interfere With Therapeutic Drug Monitoring of 12 Commonly Monitored Drugs Using Immunoassays

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St. John's wort, a popular herbal remedy for depression, is known to interact with many Western drugs because of the ability of its components to induce liver enzymes. Lower concentrations of various drugs due to increased clearance have been reported. Because immunoassays are commonly used in clinical laboratories for therapeutic drug monitoring, we studied the potential interference of St. John's wort with commonly monitored therapeutic drugs. Drug-free serum pools were supplemented with St. John's wort to achieve in vitro St. John's wort concentrations mimicking in vivo concentrations after both recommended use and overdose. Concentrations of digoxin, tricyclic antidepressants (TCAs), phenytoin, carbamazepine, theophylline, valproic acid, quinidine, phenobarbital, procainamide, and N-acetyl procainamide were measured in serum. Pooled serum specimens from patients who were taking a particular drug

were also supplemented in vitro with concentrations of St. John's wort to investigate whether observed concentrations changed after supplementation with St. John's wort. The effect of St. John's wort on cyclosporine and tacrolimus (FK 506) was studied in whole blood. We found no significant interference from St. John's wort with any assay studied. Moreover, when drug-free serum was supplemented with very high concentrations of hypericin (2 µg/mL) and hyperforin (2 µg/mL) pure standard, we observed no apparent drug level with any immunoassay. The presence of both hypericin and hyperforin was also confirmed by thin layer chromatography (TLC) in both preparations of St. John's wort. We conclude that immunoassays may be used to measure levels of therapeutic drugs in patients who self-medicate with St. John's wort. *J. Clin. Lab. Anal.* 20:62–67, 2006.

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INTRODUCTION

St. John's wort is a popular herbal antidepressant in the United States. According to a study on the use of herbal remedies, the most commonly used herb is ginseng (20%) followed by echinacea (19%), ginkgo biloba (15%), and St. John's wort (14%) (1). The most widely commercially sold St. John's wort preparation in the United States is alcoholic extract of hypericum, a perennial aromatic shrub with bright yellow flowers that bloom from June to September. The flowers are believed to be most abundant and brightest around June 24th, the day traditionally believed to be the birthday of St. John the Baptist (hence the name St. John's wort). The active components of St. John's wort induce

cytochrome P 450 mixed-function oxidase (CYP3A4), the enzyme system that is responsible for the metabolism of many drugs by the liver. St. John's wort also induces P-glycoprotein drug transporter in a clinically relevant manner, and thus reduces the efficacy of drugs for which hepatic metabolism may not be the major pathway of clearance. Hyperforin, a constituent of St. John's wort,

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is probably responsible for inducing CYP3A4 through activation of a nuclear steroid/pregnane and xenobiotic receptor. Another component, hypericin, may activate P-glycoprotein. Therefore, self-medication with St. John's wort may cause treatment failures due to the increased clearance of many prescribed drugs, including immunosuppressants (cyclosporine and tacrolimus), HIV protease inhibitors, HIV non-nucleoside reverse transcriptase inhibitors metabolized via CYP3A4, and antineoplastic drugs such as irinotecan and imatinib mesylate (2–4). Increased clearance of oral contraceptives has also been reported. Therefore, the use of St. John's wort may interfere with the effectiveness of oral contraceptives (5). The use of St. John's wort is frequently unrecognized, and may have an important influence on the effectiveness and safety of drug therapy during a hospital stay (6).

Barone et al. (7) reported two cases in which renal transplant recipients began to self-medicate with St. John's wort. Both patients experienced subtherapeutic concentrations of cyclosporine, and one patient developed acute graft rejection due to a low cyclosporine level. In both patients, termination of the use of St. John's wort returned their cyclosporine concentrations to therapeutic levels. Interaction between St. John's wort and cyclosporine is well documented in the literature. Bauer et al. (8) concluded that St. John's wort caused a rapid and significant reduction in plasma cyclosporine concentrations, as well as a substantial alteration in cyclosporine metabolite kinetics. Recently Mai et al. (9) reported that the hyperforin content of St. John's wort determines the magnitude of the interaction between St. John's wort and cyclosporine. A significant reduction in the area under the curve (AUC) for FK 506 (tacrolimus) was also observed in 10 stable renal transplant patients who were taking St. John's wort. Interestingly, no interaction was observed with another immunosuppressant, mycophenolic acid (10,11).

St. John's wort reduced the AUC of the HIV-1 protease inhibitor indinavir by a mean of 57% and decreased the extrapolated trough by 81%. A reduction in indinavir exposure of this magnitude could lead to treatment failure (12). Busti et al. (13) reported that atazanavir therapy could also be affected by simultaneous use of St. John's wort. Coadministration of lopinavir/ritonavir with St. John's wort is not recommended because it can substantially reduce lopinavir plasma concentrations (14).

A case report describing an interaction between St. John's wort and theophylline has also been published (15). A patient who was taking 300 mg of theophylline twice daily required a dosage increase of 800 mg twice daily after she began to take St. John's wort to maintain

her theophylline at a serum level of 9.2 µg/mL. Seven days after discontinuation of St. John's wort her theophylline level increased to 19.6 µg/mL. However, Morimoto et al. (16) recently observed no significant change in the pharmacokinetics of theophylline in plasma after 15 days of treatment with St. John's wort in 12 volunteers. A reduced plasma level of methadone was also observed in the presence of St. John's wort, resulting in the reappearance of withdrawal symptoms (17). Clearance of imatinib mesylate is also increased due to administration of St. John's wort and can compromise imatinib's clinical efficacy (18). Sugimoto et al. (19) reported that St. John's wort significantly reduced the plasma concentration of the cholesterol-lowering drug simvastatin, which is metabolized by CYP3A4 in the intestinal wall and liver. However, St. John's wort did not influence the plasma pravastatin concentration. St. John's wort has no effect on the pharmacokinetics of carbamazepine in healthy volunteers (20).

Interaction between St. John's wort and digoxin is of clinical significance. Johnne et al. (21) reported that 10 days' use of St. John's wort could result in a decrease of trough serum digoxin concentrations by 33% and peak digoxin concentrations by 26%. Digoxin is a substrate for P-glycoprotein, which is induced by St. John's wort. Durr et al. (22) also confirmed lower digoxin concentrations in healthy volunteers who concurrently took St. John's wort. The dose and preparation of St. John's wort also affect the pharmacokinetics of digoxin (23). Tannergren et al. (24) recently reported that repeated administration of St. John's wort significantly decreases the bioavailability of R- and S-verapamil. This effect is caused by induction of first-pass metabolism by CYP3A4, most likely in the gut.

Immunoassays are commonly used for therapeutic drug monitoring. Because St. John's wort is a crude extract of a plant, some components of the mixture may interfere with immunoassays used for drug measurement. Interference of the Chinese medicines Chan Su, DanShen, and ginseng in serum digoxin measurement by immunoassays has been extensively studied (25–30). However, no study has addressed the suitability of immunoassays for therapeutic drug monitoring in patients who are also self-medicating with St. John's wort. Here we report our findings on the suitability of immunoassays for determining concentrations of commonly monitored drugs in the presence of St. John's wort.

MATERIALS AND METHODS

Pure hypericin standard and hyperforin standard (1 mg/mL in methanol) were purchased from Sigma Chemical Company (St. Louis, MO). Two different

St. John's wort preparations were used. The first brand (brand 1) was a liquid extract of flower bulbs manufactured by Gaia Herbs Inc. (Brevard, NC). The second product (brand 2) was 300 mg of dried powder of the extract (per capsule) manufactured by Nature's Bounty Inc. (Bohemia, NY). Assay kits for several drugs were obtained from Abbott Laboratories (Abbott Park, IL) and assays were run on an AxSYM analyzer. For the fluorescence polarization immunoassay of digoxin we used a TDx/FLX analyzer, also obtained from Abbott Laboratories. Other drugs were analyzed using the Hitachi P 800 modular system and assay kits from Roche Diagnostics (Indianapolis, IN).

The liquid extract of St. John's wort was used directly for supplementation with drug-free serum (10 or 20 μ L/mL of serum). However, to avoid any matrix problem we added the extract to a test tube. The organic part of the extract (alcohol) was evaporated under a gentle stream of nitrogen at room temperature, and the residue was reconstituted with drug-free serum. For the second product (brand 2) we prepared a standard solution by dissolving 250 mg of dry powder in 10 mL of absolute ethanol. After the solution was properly mixed and the undissolved residue was allowed to remain in the solution overnight, we centrifuged the specimen. The clear supernatant was used for supplementation. Then concentrations of phenytoin, valproic acid, carbamazepine, quinidine, theophylline, and tricyclic antidepressants (TCAs) were measured using appropriate fluorescence polarization immunoassay (FPIA) assays on the AxSYM analyzer. We measured digoxin concentrations using both the FPIA assay on the TDx/FLX analyzer and the microparticle enzyme immunoassay (MEIA) assay on the AxSYM analyzer. Concentrations of procainamide, N-acetyl procainamide, and phenobarbital were measured using the Hitachi P 800 modular system and immunoassay kits marketed by Roche Diagnostics (Indianapolis, IN). Whole-blood concentrations of cyclosporine were measured using the FPIA assay and TDx/FLX analyzer, while concentrations of FK 506 were measured using the MEIA assay and IMX analyzer (Abbott Laboratories, Abbott Park, IL).

In a second set of experiments we supplemented serum pools (two pools each for every drug studied) prepared from patients who were taking digoxin, theophylline, phenytoin, carbamazepine, valproic acid, phenobarbital, procainamide, quinidine, or TCA with 10–20 μ L of St. John's wort (brands 1 and 2), and again measured the appropriate drug concentrations using the same immunoassay for comparison. Whole-blood pools containing cyclosporine or FK 506 were also supplemented with St. John's wort, and cyclosporine or FK 506 concentrations were also measured post supplementa-

tion. Such specimens are routinely submitted to our clinical laboratory for therapeutic drug monitoring. We used leftover specimens after we performed and reported all tests to the ordering clinicians, and then saved the specimens for 1 week as required by our laboratory protocol. These specimens would otherwise have been discarded.

In another experiment, drug-free serum was supplemented with pure hyperforin and hypericin standard. Commercially available hypericin was dissolved in methanol to produce a stock solution of hypericin (1 mg/mL) and then 20 μ L of stock solution were added to a test tube. Then 20 μ L of standard solution of hyperforin (1 mg/mL) were also added to the test tube and the organic phase was slowly evaporated to dryness under a gentle stream of nitrogen at room temperature. The dry residue was reconstituted with 10 mL of drug-free serum to achieve final hypericin and hyperforin concentrations of 2 μ g/mL each. Then the apparent concentrations of digoxin, TCA, phenytoin, carbamazepine, theophylline, valproic acid, quinidine, phenobarbital, procainamide, and N-acetyl procainamide were measured using appropriate immunoassays. We also added 10 μ L of hypericin standard and 10 μ L of hyperforin standard in another test tube. After the organic phase was evaporated, the residue was constituted with 5 mL of whole blood, and the apparent cyclosporine and tacrolimus (FK 506) concentrations were measured by the appropriate immunoassay.

To confirm the presence of hypericin and hyperforin in the commercially available St. John's wort used in this study, we performed thin layer chromatography (TLC) analysis using silica gel plates (Whatman Partisil silica gel 60 A⁰, layer thickness = 250 μ m, with fluorescent indicator; Fischer Scientific, Houston, TX). The developing solvent was composed of n-heptane/acetone/t-butyl methyl ether/96% acetic acid (33:35:30:2 by vol). Liquid extract of St. John's wort (brand 1) was used directly for spotting on the plate, while the second product was dissolved in absolute ethyl alcohol (25 mg/mL) and then spotted on the plate. The hypericin or hyperforin standard was also spotted on the plate for comparison. After the plates were developed, excess solvent was allowed to dry at room temperature and then spots were visualized under UV light as described by Orth et al. (31). After we visualized the spots under UV light, we sprayed the plates with a charring agent (0.5% α -naphthol, 5% sulfuric acid in absolute ethanol) followed by heating in order to further observe the spots.

Statistical analysis was performed with the use of Student's t-test, two-tailed. We considered any difference significant only at 95% confidence or higher ($P < 0.05$).

RESULTS AND DISCUSSION

When aliquots of drug-free serum (or drug-free whole blood for cyclosporine and tacrolimus) were supplemented with various concentrations of St. John's wort (both brands), we observed no apparent drug concentrations for any of the drugs tested. The observed values were always lower than the sensitivity of the assay, indicating that St. John's wort does not interfere with any of the immunoassays commonly employed for therapeutic drug monitoring in clinical laboratories. We also used a much higher *in vitro* St. John's wort concentration (20 µL/mL) to ensure that even under worst-case conditions St. John's wort does not interfere with these 12 commonly monitored therapeutic drugs using immunoassays. The concentrations we selected for this study were much higher than expected from *in vivo* concentrations of St. John's wort after recommended use and even severe overdose.

Because the cross-reactivity of an agent should be tested in the presence of the primary analyte (32), we also added St. John's wort extract to serum pools prepared from patients who were taking a particular drug. Again we observed no significant increases or decreases (<10% changes in concentration) in drug concentrations compared to the original values. For example, in a serum pool prepared from patients receiving phenytoin, the concentration of phenytoin was 16.4 µg/mL (SD = 0.18, n = 3). In the presence of 10 µL/mL of St. John's wort extract (brand 1) the observed concentration was 16.2 µg/mL (SD = 0.08, n = 3), a statistically nonsignificant change. In the presence of 20 µL/mL of extract the observed value was 15.9 µg/mL (SD = 0.15, n = 3), which was not statistically different from the original value of 16.4 µg/mL. Similarly, in a serum pool prepared from patients taking theophylline, the original theophylline concentration was 15.5 µg/mL (SD = 0.28, n = 3). In the presence of 10 µL/mL of St. John's wort extract (brand 1), the observed value was 15.8 µg/mL, which was not significantly different from the original value. We also observed no change in concentrations of valproic acid, carbamazepine, procainamide or its metabolite N-acetyl procainamide, quinidine, or digoxin in the presence of St. John's wort.

We observed no significant change in total TCA concentrations in the presence of St. John's wort. For example, in TCA pool 1 containing 160.7 ng/mL of TCA, the observed TCA in the presence of 10 µL/mL of St. John's wort (brand 1) was 158.8 ng/mL (SD: 3.1, n = 3), and in the presence of 20 µL/mL of St. John's extract it was 163.2 ng/mL (SD: 3.6, n = 3). None of these values were significantly different from the original

TABLE 1. Effect of adding St. John's wort to serum pools prepared from patients taking tricyclics on the TCA measurement by FPIA

Specimen	St. John's wort added	TCA, ng/mL, Mean (SD), n = 3
TCA Pool 1	None	160.7 (2.5)
TCA Pool 1	10 µL extract (Brand 1)	158.8 (3.1)
TCA Pool 1	20 µL extract (Brand 1)	163.2 (3.6)
TCA Pool 1	10 µL extract (Brand 2)	154.8 (4.1)
TCA Pool 1	20 µL extract (Brand 2)	160.2 (2.8)
TCA Pool 2	None	261.4 (10.8)
TCA Pool 2	10 µL extract (Brand 1)	254.7 (3.6)
TCA Pool 2	20 µL extract (Brand 1)	251.1 (7.6)
TCA Pool 2	10 µL extract (Brand 2)	254.7 (4.1)
TCA Pool 2	20 µL extract (Brand 2)	251.1 (4.8)

TCA concentration of pool 1. We observed similar results with brand 2 of the St. John's wort product, as well as with TCA pool 2 (Table 1).

When aliquots of whole-blood specimens prepared from patients receiving cyclosporine or tacrolimus were further supplemented with St. John's wort (both brands 1 and 2), we observed a <10% change in whole-blood drug concentrations, indicating that St. John's wort has no significant influence on whole-blood-based immunoassays for cyclosporine and tacrolimus.

The concentrations of hypericin are very low after consumption of St. John's wort. Schempp et al. (33) reported that after an oral dose of St. John's wort, the combined mean level of hypericin and pseudohypericin was 42 ng/mL. After steady state was reached, the total hypericin level was only 12.5 ng/mL. Staffeldt et al. (34) reported that the maximum plasma levels of hypericin reached 1.5 ng/mL with a 300-mg daily dose, 4.1 ng/mL with a 900-mg daily dose, and 14.2 ng/mL with a 1,800-mg daily dose of dried hypericum extract in healthy volunteers. Agrosi et al. (35) observed a peak plasma hyperforin level of 168.35 ng/mL after consumption of a soft gelatin formulation and 84.25 ng/mL after consumption of hard gelatin capsules. Vitiello et al. (36) were unable to detect any hyperforin level in 17 out of 97 volunteers who took St. John's wort.

To investigate the worst-case scenario, we supplemented drug-free serum with 2 µg/mL of hypericin pure standard and 2 µg/mL of hyperforin standard, and then measured apparent drug levels with the appropriate immunoassays. We observed no measurable concentration of digoxin, TCA, phenytoin, carbamazepine, theophylline, valproic acid, quinidine, phenobarbital, procainamide, or N-acetyl-procainamide even in the presence of such high concentrations of the active ingredients of St. John's wort. This indicates that it is

unlikely that any St. John's wort preparation will interfere with the immunoassays used in clinical laboratories for therapeutic drug monitoring of these drugs (N-acetyl procainamide is the active metabolite of procainamide). Moreover, when we supplemented whole blood with both hypericin and hyperforin, we observed no apparent cyclosporine or tacrolimus (FK 506) level, indicating that such assays should not be affected by St. John's wort. Hypericin is metabolized via hepatic glucuronidization followed by biliary excretion. It is unlikely that the hypericin metabolite would interfere with any immunoassays we studied for therapeutic drug monitoring. However, in vivo data are needed before any definite conclusions can be drawn.

Both preparations of St. John's wort used in this study showed the presence of the active ingredients hypericin and hyperforin by TLC analysis. According to the package insert, brand 1 contained 0.5% hypericin (total hypericin content in a single dose of 30 drops = 0.2 mg) and brand 2 contained 0.3% hypericin (total hypericin in a single 300 mg capsule = 0.9 mg). The package insert did not mention the hyperforin content. TLC analysis of both products confirmed the presence of hypericin, since the retention factor of hypericin as found in brands 1 and 2 matched the retention factor of the hypericin standard. Similarly, the retention factor of hyperforin as found in both products agreed with the retention factor of the hyperforin standard, indicating that both products used in this study contained the desired active ingredients of St. John's wort.

Because there is no commercially available immunoassay to measure concentrations of anti-HIV drugs, and only HPLC combined with mass spectrometry is used to monitor such drugs, it is very unlikely that St. John's wort would interfere with such a sophisticated method. Our study clearly indicates that St. John's wort does not interfere with immunoassays used to monitor common therapeutic drugs. Therefore, reduced drug concentrations due to the use of St. John's wort truly reflect drug-herb interactions and increased clearance of therapeutic drugs. Moreover, such assays can also be used to study interactions between St. John's wort and Western drugs in research laboratories.

We conclude that St. John's wort does not affect the measurement of 12 commonly monitored therapeutic drugs using immunoassays. Moreover, high concentrations of hypericin and hyperforin, the active ingredients of St. John's wort, did not interfere in vitro with any of the 12 immunoassays used in this study. However, in vivo data are needed before we can reach a final conclusion regarding the suitability of such immunoassays to monitor therapeutic drug levels in patients who self-medicate with St. John's wort.

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