

# Toxicology News

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## Herbal Medicines can Cause Significant Drug Interactions

By Amitava Dasgupta

**H**erbal medicines are readily available without prescriptions from herbal stores in the United States. In Europe, herbal medicines represent an important pharmaceutical market with annual sales of \$7 billion. In the United States, herbal medicine sales increased from \$200 million in 1988 to over \$3.3 billion in 1997. The common public conception that anything natural is safe is wrong, and herbal medicines, like Western medicines, can be toxic and have significant side effects. Incorrect use or overuse of herbal medicines can even cause death.

### Regulatory issues

Under U.S. law, substances categorized as medicines must be proven to be safe before being released to the market, but herbal products do not necessarily fall under the category of medicines or drugs. As long as they are not marketed for the prevention or treatment of a disease, herbal products can be classified as dietary supplements, so no Food and Drug Administration approval is required. These substances are regulated by the Dietary Supplement Health and Education Act of 1994, which is much more lax than the laws covering drugs.

### Drug-herb interactions

The mechanisms of drug-herb interactions can be classified in several categories:

An herbal product may increase the clearance of a Western drug, leading to an unexpected lower concentration of a therapeutic drug. St. John's wort, an herbal antidepressant, increases clearance of many Western drugs.

An herbal medicine may have a synergistic effect and increase the pharmacological activity of a Western drug or may decrease therapeutic efficacy of a drug.

An herbal medicine may displace a Western drug from serum protein, increasing free drug concentration, the pharmacologically active component of a drug.

### St. John's wort interactions

The active component of St. John's wort induces the cytochrome P450 mixed-function oxidase (CYP3A4) enzyme system responsible for the metabolism of many drugs by the liver. St. John's wort also induces the P-glycoprotein drug transporter in a clinically relevant manner, thereby reducing the efficacy of drugs in which hepatic metabolism is not the major pathway of clearance. The St. John's wort constituent hyperforin is probably responsible for the CYP3A4 induction 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 can cause treatment failures due to an increase in the clearance of many prescribed drugs. These drugs include immunosuppressants (cyclosporine and tacrolimus), HIV protease inhibitors, HIV non-nucleoside reverse transcriptase inhibitors metabolized via CYP3A4, and anti-neoplastic drugs such as irinotecan and imatinib mesylate. Increased clearance of oral contraceptives has also been reported. The unrecognized use of St. John's wort can have an important influence on the effectiveness and safety of drug therapy during a hospital stay.

Barone et al. reported two cases in which renal transplant recipients started self-medication with St. John's wort. Both patients experienced subtherapeutic concentrations of cyclosporine and one developed

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## Laboratories Must Validate Test Method Characteristics

By Brian A. Brunelli

Because the reporting of a positive drug test can have a profound impact on the individual being tested, it is imperative that each analytical procedure a laboratory uses be properly validated to ensure accuracy and legal defensibility.

Regardless of the methodology used for screening or confirmation testing, a laboratory must validate the critical performance characteristics of each test and each test system prior to implementing them and after any significant modification to the testing process. These critical performance characteristics include linearity, precision, accuracy, specificity, sensitivity, and carryover potential.

### Immunoassay method validation: linearity

Immunoassay procedures are generally considered to be non-linear in nature and typically exhibit an S-shaped curve of concentration vs. response. Reagent manufacturers optimize their formulations so the linear portion of the S-shaped response curve coincides with the intended cutoff of the assay. When a laboratory evaluates the linearity of an immunoassay, it is critical to verify that the procedure is linear around the intended cutoff. This can be accomplished by analysis of standards containing the drug of interest at concentrations above and below the intended cutoff (e.g., 0%, 50%, 75%, 125%, 150%, and 200% of the cutoff). A plot of delta absorbance units vs. concentration can be used to determine the linear portion of the curve. If multiple cutoffs are used in a single procedure, the linearity around each cutoff should be evaluated.

### Accuracy and precision

The accuracy of each immunoassay needs to be evaluated to ensure that the procedure can distinguish between potentially positive samples and negative samples. The precision must be evaluated to ensure that the results are reproducible. Accuracy and precision can be assessed simultaneously by analysis of 20 replicates of standards containing the drug of interest at 75%, 100%, and 125% of cutoff. For the procedure to demonstrate accuracy below the cutoff, the mean response of the 75% cutoff standard plus two standard deviations (+2 SD) should not overlap with the mean response of the 100% cutoff standard. To demonstrate accuracy above the cutoff, the mean response of the 125% standard minus two standard deviations (-2 SD) should not overlap with the mean response of the 100% standard.

The precision of the procedure around the cutoff can be determined by calculating the mean, standard deviation, and coefficient of variation of the absorbance values of the 75%, 100%, and 125% standards.

To evaluate inter-run precision, the same series of 20 standards at 75%, 100%, and 125% of cutoff should be tested on five consecutive days. In most situations, an intra-run or inter-run precision of <10% is acceptable.

### Specificity and cross-reactivity

The ability of an immunoassay to distinguish between the target drug and structurally similar compounds can be expressed in terms of specificity and/or cross-reactivity. The desired degrees of specificity and cross-reactivity of an immunoassay depend on the application. Cross-reactivity can be advantageous in some situations because the analysis can be used to detect drug classes (for example, barbiturates, benzodiazepines, or opiates) instead of one specific drug within the class. Cross-reactivity can be disadvantageous in other situations because the analysis may detect drugs of no interest. For example, some amphetamine immunoassays cross-react with over-the-counter sympathomimetic amines such as pseudoephedrine, ephedrine, and phenylpropanolamine to produce a positive response, resulting in unnecessary confirmation testing and added expense.

Most reagent manufacturers publish specificity and cross-reactivity data in their package inserts, but the data may not accurately reflect the reagents' performance on all instrument types. Therefore, it is essential for each laboratory to verify the specificity and cross-reactivity of each procedure independently. They can be determined by identifying the concentration of the drug that elicits a positive response.

### Carryover

The term carryover refers to residual drug from one sample contaminating the following sample to a sufficient degree to produce a positive or measurable response. In immunoassays, carryover can be caused by insufficient washing of the pipette mechanism or by splashing between samples. To evaluate the potential for carryover, the laboratory can analyze samples with high drug concentrations followed by drug-free samples. The concentrations of drugs evaluated should reflect those concentrations observed within the routine testing population. Criteria should be established to determine the permissible carryover response and all presumptive positive specimens should be subject to confirmation.

### Parallel performance

Prior to replacing an existing immunoassay with

a new one or implementing a new type of test instrument, the laboratory should perform parallel testing between the new and former procedures to verify comparable performance. Parallel performance can be evaluated by analyzing a select number of positive and negative samples by both procedures or instruments. Any discrepancies between results should be investigated. For assays in which infrequent positive samples are observed in the routine test population, the laboratory can use spiked positive specimens. The concentrations of drugs should reflect the concentrations expected in actual specimens.

### Confirmation method validation: linearity

The dynamic linear range of a method is determined by identifying the range of concentrations that produce a response that is directly proportional to the concentration of drug in a sample. The upper limit of linearity (ULOL) is defined as the highest concentration of drug that can be detected while meeting qualitative and quantitative criteria. The limit of quantitation (LOQ) is defined as the lowest concentration of drug that can be detected while meeting qualitative and quantitative criteria. The ULOL and LOQ can be determined experimentally by the analysis of a series of standards above and below the cutoff. The observed concentration of each standard should be within  $\pm 20\%$  of the target concentration to be acceptable. The laboratory can also use the data to calculate a regression line by the method of least squares and evaluate the correlation coefficient of the line as an indicator of linearity. A correlation coefficient of  $\geq 0.975$  is evidence of acceptable method linearity.

### Limit of detection

The limit of detection (LOD) is the lowest concentration of analyte that can be detected with qualitative acceptance criteria (such as chromatography, retention time, ion ratios, etc.). The laboratory can apply an empirical approach for determination of the LOD by analyzing a series of standards of decreasing concentrations until the lowest concentration is found.

### Specificity/interference

As part of the confirmation method validation process, it is critical to verify that the procedure is able to accurately discriminate between the drug of interest and structurally similar compounds, including metabolic precursors and byproducts. This is of particular concern for the confirmation procedures for opiates and amphetamines because many synthetic analogs exist. To verify specificity and lack of interference, the laboratory should analyze samples containing potentially interfering substances in the

presence and absence of the drug of interest and apply both qualitative and quantitative acceptance criteria. It is recommended that, at a minimum, the opiates, 6-acetylmorphine, and amphetamines confirmation procedures be evaluated for potential interference from several drugs in the presence of the analyte at 40% of the cutoff and without the analyte.

The opiates (codeine and morphine) procedure should be checked for interference from hydrocodone, hydromorphone, oxycodone, oxymorphone, and norcodeine at 5,000 ng/mL. The 6-acetylmorphine test should be checked for interference from free morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and norcodeine at 5,000 ng/mL. The amphetamines (amphetamine and methamphetamine) test should be checked with phentermine at 50,000 ng/mL and phenylpropanolamine, ephedrine, and pseudoephedrine at 1 mg/mL.

### Carryover

In confirmation testing, carryover can be caused by a previously analyzed sample that contaminates the injection syringe, wash reservoir solvents, injection port, or analytical column. Each confirmation method must be evaluated for carryover potential to ensure that quantitative results are not falsely elevated. Carryover can be evaluated by analysis of standards containing high drug concentrations followed by drug-free samples. The laboratory must have specific criteria for evaluation of the drug-free sample for potential carryover. An acceptable criterion is to document that there is no drug above the established LOD in a drug-free sample that follows a sample with a high concentration.

### Accuracy and precision

The accuracy of an analytical method is the degree of agreement of test results generated by the method to the true value. The precision is the degree of agreement among test results when the procedure is applied repeatedly to samples of the same standard concentration. Both accuracy and precision can be simultaneously assessed by analysis of 20 replicates of standards containing the drug of interest at 40%, 100%, and 125% of cutoff. The accuracy of the procedure can be evaluated by comparing the experimental results with the target concentration. The precision of the procedure can be determined by calculating the mean, standard deviation, and coefficient of variation of the 40%, 100%, and 125% standards. When evaluating inter-run precision, the same series of 20 standards at 40%, 100%, and 125% of cutoff should be tested on five consecutive days. In most situations, an intra-run or inter-run coefficient of variation of  $< 10\%$  is acceptable.

## Conclusion

Proper method validation for new or revised immunoassay and chromatographic confirmation procedures is a key to a quality drug-testing program. Data for performance characteristics such as linearity, accuracy, precision, specificity, and carryover potential provide evidence that the method does what it is intended to do, reliably and reproducibly.

## Suggested Reading

1. Clinical and Laboratory Standards Institute. Gas chromatography/mass spectrometry (GC/MS) confirmation of drugs; approved guideline. NCCLS document C43-A. Wayne, Pennsylvania: CLSI, 2002.
2. Clinical and Laboratory Standards Institute. Urine drug testing in the clinical laboratory; approved guideline. NCCLS document T/DM08-A. Wayne, Pennsylvania: CLSI, 1999.
3. Huber L. Good laboratory practice: a primer for high-performance liquid chromatography, capillary electrophoresis and UV-visible spectroscopy. Germany: Hewlett Packard Co., 1993;46–53.
4. U.S. Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Division of Workplace Programs. Mandatory guidelines for federal workplace drug testing programs. Fed Reg 1997;69:19644–73.

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## Herbal Medicines

*Continued from page 1*

acute graft rejection due to a low cyclosporine level. When the patients terminated their use of St. John's wort, their cyclosporine concentrations returned to therapeutic levels (1).

The interaction between St. John's wort and cyclosporine is well-documented in the literature. Bauer et al. concluded that St. John's wort caused a rapid and significant reduction in plasma cyclosporine concentrations (2). Recently, Mai et al. reported that the hyperforin content of St. John's wort determined the magnitude of the interaction between St. John's wort and cyclosporine (3). A significant reduction in the area-under-the-concentration-over-time curve (AUC) for tacrolimus was also observed in 10 stable renal transplant patients receiving St. John's wort. Interestingly, no interaction was observed with another immunosuppressant, mycophenolic acid (4).

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 (5). Busti et al. reported that atazanavir therapy can also be affected by St. John's wort (6). St. John's wort can also substantially reduce lopinavir plasma concentrations.

## More St. John's wort interactions

An interaction between St. John's wort and theophylline has also been reported. When a patient taking 300 mg of theophylline twice daily began taking St. John's wort, she required a dosage increase of 800 mg twice daily to maintain her theophylline serum level at 9.2  $\mu\text{g/mL}$ . Seven days after discontinuation of St. John's wort, her theophylline level increased to 19.6  $\mu\text{g/mL}$  (7). A reduced plasma level of methadone was also observed in the presence of St. John's wort, resulting in the reappearance of withdrawal symptoms (8). St. John's wort also results in increased clearance of imatinib mesylate, compromising the drug's clinical efficacy (9). St. John's wort also interacts significantly with oral contraceptives (10). Sugimoto et al. reported that St. John's wort significantly reduces plasma concentrations of the cholesterol-lowering drug simvastatin, which is metabolized by CYP3A4 in the intestinal wall and liver (11).

Johne et al. reported that using St. John's wort for 10 days could result in a decrease in 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 (12). Tannergren et al. reported that repeated administration of St. John's wort significantly decreases bioavailability of R- and S-verapamil. This effect is caused by induction of first-pass metabolism by CYP3A4, most likely in the gut (13).

Herbal remedies are not prepared following rigorous pharmaceutical standards, so wide variations in the amount and strength of the active components have been reported in various commercial preparations. Draves and Walker reported that in commercial tablets the percentage of active components varied from 31.3% to 80.2% of active ingredients listed on the bottles' labels (14). Therefore, different brands of products can vary widely in the magnitude of their effects.

## Warfarin interactions

Warfarin acts by antagonizing the cofactor function of vitamin K. Variability in the anticoagulant response to warfarin is an ongoing clinical dilemma. Although clinical efficacy of warfarin varies with vitamin K intake, genetic polymorphisms that may modulate expression of CYP2C9, and the isoform mediating clearance of S-warfarin, several warfarin-herb interactions also may significantly

affect warfarin therapy. St. John's wort has the potential to diminish warfarin's anticoagulation effect by increasing clearance through inducing CYP2C9 (15). Another report indicates that St. John's wort increases clearance of both R- and S-warfarin, but that ginseng has no effect (16).

The anticoagulant effect of warfarin should increase if combined with coumarin-containing herbal remedies, such as boldo, fenugreek, or dong quai, or with anti-platelet herbs, such as danshen, garlic, or ginkgo biloba. Conversely, substances that contain vitamin K, such as green tea, should antagonize the anticoagulant effect of warfarin (16–18). In a patient treated with warfarin for atrial fibrillation, the international normalization ration (INR) was increased when he started taking the coumarin-containing herbal products boldo and fenugreek. After discontinuation of herbal supplements, his INR returned to normal after one week (19). Increased anticoagulation due to interaction between warfarin and danshen has been reported (20, 21). Two cases of increased INR were reported after coadministration of curbicin (a preparation containing saw palmetto, pumpkin, and vitamin E). The INR normalized after discontinuation of the herbal supplement (22). Two cases of increased INR were mentioned in patients taking garlic who had been previously stabilized on warfarin (22, 23). A likely mechanism is an additive effect because garlic has anti-platelet activity.

Samuels recently discussed the effects of herbal medicines on anticoagulation therapy (24). Major drug-herb interactions are summarized in Table 1.

### Herbal medicines and perioperative patients

The use of herbal remedies by perioperative patients is a significant problem because the herbs can interact with anesthetics as well as lead to bleeding during and after surgery. A recent study reported that 57% of survey respondents scheduled for elective surgery used herbal supplements at some point in their lives. More interestingly, 38% of respondents used herbal supplements in the past two years, and 17% used herbal supplements during the month of their surgery (25).

Ang-Lee et al. recommend that garlic and ginseng be discontinued at least seven days prior to surgery because both herbs have been reported to cause bleeding (26). Ginkgo biloba should also be discontinued three days prior to surgery because ginkgo also inhibits platelet aggregation, causing bleeding. Kava should be discontinued at least 24 hours before surgery because kava can increase the sedative effect of anesthetics. Ma huang (ephedra) should be discontinued 24 hours prior to surgery

because it increases blood pressure and heart rate. St. John's wort should be discontinued five days prior to surgery because it induces cytochrome P450, which is involved in the metabolism of many drugs. Therefore, St. John's wort can significantly reduce the concentrations of many drugs, such as cyclosporine, warfarin, and steroids. This is particularly important for transplant surgery because cyclosporine is used as an immunosuppressant.

The American Society of Anesthesiologists, however, has a much simpler recommendation: All herbal supplements should be discontinued two to three weeks before elective surgery.

### References

1. Barone GW, Gurley BJ, Ketel BL, Abul-Ezz SR. Herbal supplements; a potential for drug interactions in transplant recipients. *Transplantation* 2001;71:239–41.
2. Bauer S, Stormer E, Johnne A, Kruger H, Budde K, Neumayer HH, et al. Alterations in cyclosporin A pharmacokinetics and metabolism during treatment with St. John's wort in renal transplant patients. *Br J Clin Pharmacol* 2003;55:203–11.
3. Mai I, Bauer S, Perloff ES, Johnne A, Uehleke B, Frank B, et al. Hyperforin content determines the magnitude of the St. John's wort-cyclosporine drug interaction. *Clin Pharmacol* 2004;76:330–40.
4. Mai I, Stormer E, Bauer S, Kruger H, Budde K, Roots I. Impact of St. John's wort treatment on the pharmacokinetics of tacrolimus and mycophenolic acid in renal transplant patients. *Nephrol Dial Transplant* 2003;18:819–22.
5. Piscitelli SC, Burstein AH, Chait D, Alfaro RM, Fallon J. Indinavir concentrations and St. John's wort. *Lancet* 2000;355:547–8.
6. Busti AJ, Hall RG, Margolis DM. Atazanavir for the treatment of human immunodeficiency virus infection. *Pharmacotherapy* 2004;24:1732–47.
7. Nebel A, Schneider BJ, Kroll DJ. Potential metabolic interaction between St. John's wort and theophylline. *Ann Pharmacother* 1999;33:502.
8. Eich-Hochli D, Oppliger R, Golay KP, Baumann P, Eap CB. Methadone maintenance treatment and St. John's wort: a case study. *Pharmacopsychiatry* 2003;36:35–7.
9. Smith P. The influence of St. John's wort on the pharmacokinetics and protein binding of imatinib mesylate. *Pharmacotherapy* 2004;24:1508–14.
10. Murphy PA, Kern SE, Stanczyk FZ, Westhoff CL. Interaction of St. John's wort with oral contraceptives: effects on the pharmacokinetics of norethindrone and ethinyl estradiol, ovarian activity and breakthrough bleeding. *Contraception* 2005;71:402–8.
11. Sugimoto K, Ohmori M, Tsuruoka S, Nishiki K, Kawaguchi A, Harada K, et al. Different effects of St. John's wort on the pharmacokinetics of simvastatin and pravastatin. *Clin Pharmacol Ther* 2001;70:518–24.

12. Johne A, Brockmoller J, Bauer S, Maurer A, Langheinrich M, Roots I. Pharmacokinetic interaction of digoxin with an herbal extract from St John's wort (*Hypericum perforatum*). *Clin Pharmacol Ther* 1999;66:338–45.
13. Tannergren C, Engman H, Knutson L, Hedeland M, Bondesson U, Lennernas H. St John's wort decreases the bioavailability of R- and S-verapamil through induction of the first pass metabolism. *Clin Pharmacol Ther* 2004;75:298–309.
14. Draves AH, Walker SE. Analysis of hypericin and pseudohypericin content of commercially available St. John's wort preparation. *Can J Clin Pharmacol* 2003;10:114–8.
15. Greenblatt DJ, von Moltke LL. Interaction of warfarin with drugs, natural substances and food. *J Clin Pharmacol* 2005;45:127–32.
16. Jiang X, Williams KM, Liauw WS, Ammit AJ, Roufogalis BD, Duke CC, et al. Effect of St. John's wort and ginseng on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects. *Br J Clin Pharmacol* 2004;57:592–9.
17. Heck AM, DeWitt BA, Lukes AL. Potential interactions between alternative therapies and warfarin. *Am J Health-Syst Pharm* 2000;57:1221–7.
18. Izzo A, Di Carlo G, Borrelli F, Ernst E. Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction. *Int J Cardiol* 2005;98:1–14.
19. Lambert JP, Cormier A. Potential interaction between warfarin and boldo-fenugreek. *Pharmacotherapy* 2002;21:509–12.
20. Tam LS, Chan TY, Leung WK, Critchley JA. Warfarin interaction with Chinese traditional medicines: danshen and methyl salicylate medicated oil. *Aust N Z J Med* 1995;25:238.
21. Yu CM, Chan JC, Sanderson JE. Chinese herbs and warfarin potentiation by danshen. *J Intern Med* 1997;25:337–9.
22. Yue QY, Jansson K. Herbal drug and anticoagulant effect with and without warfarin: possibly related to vitamin E component. *J Am Geriatr Soc* 2001;49:838.
23. Sunter WH. Warfarin and garlic. *Pharm J* 1992;246:772.
24. Samuels N. Herbal remedies and anticoagulant therapy. *Thromb Haemost* 2005;93:3–7.
25. Miller LG. Herbal medicinals: selected clinical considerations focusing on known or potential drug–herb

**Table 1. Common Drug–Herb Interactions**

Herbal Product	Interacting Drug	Comments
Ginseng	Warfarin	May decrease effectiveness of warfarin
St. John's wort	Paxil	Lethargy, incoherence, nausea
	Digoxin	Decreased AUC and decreased peak and trough concentrations of digoxin, may reduce effectiveness of digoxin
	Cyclosporine/FK 506	Lower cyclosporine/FK 506 concentrations due to increased clearance, may cause transplant rejection
	Theophylline	Lower concentration decreases the efficacy of theophylline
	Indinavir, lopinavir	Lower concentrations may cause treatment failure in ritonavir, atazanavir patients with HIV
Ginkgo biloba	Statins	Reduced plasma concentration of simvastatin but no effect on pravastatin
	Irinotecan, Imatinib	Reduced efficacy
	R- and S-Verapamil	Increased clearance
	Oral contraceptives	Lower concentration may cause failed birth control
Kava	Aspirin	Bleeding because ginkgo can inhibit platelet-activating factor
	Warfarin	Hemorrhage
	Thiazide	Hypertension
Kava	Alprazolam	Additive effects with central nervous system depressants, alcohol
Garlic	Warfarin	Increased effectiveness of warfarin, bleeding
Ginger	Warfarin	Increased effectiveness of warfarin, bleeding
Feverfew	Warfarin	Increased effectiveness of warfarin, bleeding
Dong quai	Warfarin	Dong quai contains coumarin and increases INR for warfarin, bleeding
Danshen	Warfarin	Increased effectiveness of warfarin due to reduced elimination
Comfrey	Phenobarbital	Increased metabolism of comfrey, producing a lethal metabolite from pyrrolizidine, severe hepatotoxicity
Evening primrose oil	Phenobarbital	May lower seizure threshold and require dose increase

interactions. *Arch Intern Med* 1998;158:2200–11.  
26. Ang-Lee M, Moss J, Yuan CS. Herbal medicines and perioperative care. *JAMA* 2001;286:208–16.

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## Serotonin Syndrome Presents A Diagnostic Challenge to EDs

By Michele Fowler

Serotonin is an alkylamine that acts centrally as a neurotransmitter. It is involved with mood, personality, affect, appetite, temperature regulation, pain perception, and other basic functions. Serotonin is not required for these functions, but it helps to modulate their quality. There are several serotonin receptor subtypes, with the most important being 5HT1 and 5HT2 (1).

Selective serotonin reuptake inhibitors (SSRIs) are a relatively new class of medications. Introduced in the early 1980s, they have largely replaced tricyclic antidepressants as first-line therapy for depression in the United States. There are currently six SSRIs on the market: fluoxetine (Prozac), citalopram (Celexa), escitalopram (Lexapro), fluvoxamine (Luvox), paroxetine (Paxil), and sertraline (Zoloft).

They have a satisfactory safety profile with few unwanted pharmacologic actions. SSRIs reach peak plasma concentrations three to eight hours after therapeutic doses. They are highly lipophilic, are highly bound to plasma proteins, and have large volumes of distribution. They are metabolized by the liver by the cytochrome P450 isoenzyme system (2).

### Overdose

Cases of acute overdose are common because of the significant number of people who are prescribed these medications, but overdoses are usually mild and seldom fatal. Deaths related to SSRIs are usually linked to polysubstance ingestions rather than isolated SSRI ingestion. Close to half of the adults who overdosed on SSRIs had no symptoms at all.

The most common overdose symptoms are nausea, vomiting, dizziness, blurred vision, tachycardia, and tremor. Citalopram caused QRS widening and seizures in patients taking more than 600 mg in a single ingestion. Most acute overdoses, either intentional or accidental, have very low morbidity and mortality. Supportive care, activated charcoal, and

observation are usually sufficient for most SSRI overdoses. Citalopram ingestions require longer observation because of the potential for EKG changes later in the course (2).

### Serotonin syndrome

A rare but serious and potentially fatal side effect of SSRI use is serotonin syndrome, a set of symptoms involving alterations in cognition, behavior, the autonomic nervous system, and neuromuscular activity. Serotonin syndrome was perhaps first described in the 11th century among people who had ingested rye containing *Claviceps purpurea*. This fungus produces ergot alkaloids that stimulate 5-HT1 and 5-HT2 receptors, causing serotonin-type symptoms, such as limb flexion and extension, mania, sweating, fever, and others (3).

Although the exact pathophysiology is unknown, serotonin syndrome appears to involve overstimulation of 5-HT1A receptors. It can occur with acute overdose, but most commonly occurs when a patient taking medication has a dose increase or has another serotonin agonist added to the medication regimen.

The onset of symptoms is most common in the first 24 hours after changing doses or adding another serotonergic agent. Medications such as tramadol and meperidine, which are serotonin agonists, have been implicated in the development of serotonin syndrome. Cases of serotonin syndrome have also been reported in St. John's wort users (4). Because SSRIs are prescribed so frequently, it is important to get a complete history and medication list before adding any additional ones.

Illicit drug use could potentially cause serotonin syndrome in patients taking an SSRI. Amphetamines, cocaine, MDMA, and other illicit drugs can increase synthesis and inhibit uptake of serotonin. Illicit drugs alone could theoretically induce serotonin syndrome. Patients are often not forthcoming about their illicit drug use. It is important to be diligent in inquiring about the history of drug use, and sometimes a drug screen is needed to help with the diagnosis (1).

### Clinical features

Clinical features of serotonin syndrome are confusion, agitation, myoclonus, tremor, and diarrhea. Hyperreflexia, hyperthermia, and diaphoresis are also common. Shivering is seen in serotonin syndrome, though it is not commonly seen in other hyperthermic diseases (5). Undiagnosed and untreated patients can develop rhabdomyolysis, lactic acidosis, renal and hepatic failure, and disseminated intravascular coagulation. Diagnosing this syndrome is difficult because of the nonspecific constellation of symptoms and because there are no laboratory tests for it. Diagnosis is

made by a thorough history of the patient's medications and exclusion of other disease etiologies (6).

Diagnostic criteria of serotonin syndrome include addition of serotonergic agents or a recent increase in SSRI dosing. Three of the following signs and symptoms must be present: agitation, ataxia, diaphoresis, diarrhea, hyperreflexia, hyperthermia, mental status changes, myoclonus, shivering, or tremor. A neuroleptic must not have been started or increased in dosage before symptom onset. In addition, other etiologies must be ruled out (2).

The most important treatment once the diagnosis has been made is to stop the SSRI immediately and provide supportive care. Cyproheptadine, a serotonergic antagonist, and methysergide have been used for treatment, but the therapy has not been proven. Dantrolene has been used, although it is primarily reserved for neuroleptic malignant syndrome.

Neuroleptic malignant syndrome (NMS) shares many clinical features with serotonin syndrome. NMS presents a serious emergency with a mortality rate as high as 20%. The primary differences are that NMS, which is from rapid blockade of dopaminergic neurons in the central nervous system, has more delayed onset of symptoms and has more pronounced muscle rigidity. Fortunately, serotonin syndrome is self-limiting after the offending agent has been discontinued and supportive care is provided (5).

Although serotonin syndrome is rare, it is very important to be able to identify it quickly in the emergency department. Often patients present with nonspecific symptoms and only the history can help

make the diagnosis. Rapid recognition of symptoms, discontinuation of serotonergic agents, and supportive care are usually sufficient to treat this syndrome.

## References

1. Goldfrank LR, Flomenbaum N, Lewin N, Howland MA, Hoffman R, Nelson L. Goldfrank's toxicologic emergencies, 6th ed. New York: McGraw-Hill Professional, 1998;152-5,935-41.
2. Marx JA, Hockberger RS, Walls R. Rosen's emergency medicine, 5th ed. St. Louis: C.V. Mosby, 2002;2093-103.
3. Eadie MJ. Convulsive ergotism: epidemics of the serotonin syndrome. *Lancet Neurol* 2003;2(7):429-34.
4. Birmes P, Coppin D, Schmitt L, Lauque D. Serotonin: a brief review. *CMAJ* 2003;168(11):1439-42.
5. Christensen RC. Identifying serotonin syndrome in the emergency department. *Am J Emerg Med* 2005;23(3):406-8.
6. Ganetsky M, Brush DE. Serotonin syndrome—what have we learned? *Clin Ped Emerg Med* 2005;6(2):103-8.

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