Reliability of Antidepressant Assays: a Reference Laboratory Perspective on Antidepressant Monitoring

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Chromatography (gas and liquid) and immunoassays are used for monitoring the commonly prescribed tricyclic antidepressants. Many commercially available immunoassays are known to cross react with structurally similar compounds. Chromatographic methods make it possible simultaneously to resolve and quantify amitriptyline, nortriptyline, imipramine, desipramine, trimipramine, doxepin, desmethyldoxepin, protriptyline, and maprotiline—and potentially cross-reactive compounds can be separated from the tricyclics. Immunoassays may have a valuable role in initial toxicological screening for the presence of a tricyclic-like compound, and they also may be helpful in a laboratory dedicated to a well-controlled patient group. However, 10% of our specimens contain more or different antidepressants than we are requested to analyze for. With our analysis, we are able to report which antidepressants are present, and in what concentrations. Further, in the case of a potential overdose of tricyclic, the primary purpose for early toxicological analysis is to anticipate subsequent clinical complications. Therefore, even in the case of toxicological analysis, it is important to know exactly what tricyclic antidepressant is present rather than just the semiquantitative presence of one or more structurally related compounds, because these various compounds differ markedly in their potential for adverse effects. There are too many potential, and possibly yet unknown, interactions for a reference laboratory routinely to rely on immunoassays for therapeutic drug monitoring or toxicological identification of antidepressants.

Currently, a physician can choose from many drugs for the treatment of endogenous depression. These drugs include the tricyclic antidepressants, the tetracyclic antidepressants, other related antidepressants, and unrelated compounds such as lithium and alprazolam (Xanax) (1). Here, we emphasize the monitoring of tricyclic compounds such as nortriptyline (Pamelor) and desipramine (Norpramin, Pertofrane), and tetracyclic compounds such as maprotiline (Ludionil). Concentrations achieved in serum vary greatly in different patients who are receiving the same dose. A particularly compelling case for the need to routinely monitor antidepressant concentrations because of this dramatic variability is documented by Nutter and Brunswick (2). There are also many drug–drug interactions that can further alter the relationship between dose and concentration (3–5). Clinicians and laboratorians have become increasingly aware of the importance of optimizing drug therapy by measuring the drug concentration in serum or plasma (6), including the tricyclic antidepressants (7–9). Higher concentrations of the antidepressants cause a higher incidence of mild toxicities, a higher risk for serious toxicities, and a loss of the desired antidepressant response.

A medical procedure with positive cost–benefit analyses should always be well accepted. In these times of contained hospitalization reimbursement, a procedure that permits earlier discharge from the hospital is extremely popular. Therefore therapeutic drug monitoring is increasingly used to optimize antidepressant therapy (10).

Our discussion gives a reference-laboratory perspective on using the available assays for tricyclic antidepressants to provide this therapeutic drug monitoring service, as well as on evaluation of a potential overdose of a tricyclic compound.

One problem impeding universal acceptance of monitoring antidepressant drug concentrations is the discrepancy in results generated by different laboratories (11). Much of this discrepancy can be avoided by (a) consistently monitoring either plasma or serum and not interchanging serum and plasma (12, 13), (b) not using collection tubes containing a separation gel, such as SST tubes (14), and (c) using a laboratory that frequently assays antidepressants and thus has continued competence in the assays (11). Previously the brand and type of collection tube caused large variances in measured antidepressant concentrations. The two major U.S. brands of collection tubes, Venoeject and Vacutainer, do not currently cause any problems as long as separation gels are avoided. (We believe that Becton-Dickinson deserves credit for quickly changing their product as soon as the problem with drug concentrations was identified.) The antidepressants are not the only compounds that should not be collected in tubes with separation gels. We recommend that separation gels not be used for many other compounds, including many antiepileptics (such as carbamazepine and clonazepam), neuroleptics (including chlorpromazine, fluphenazine, trifluoperazine, haloperidol, thiothixene, thioridazine, and mesoridazine), cardiac agents (such as propranolol, metoprolol, verapamil, disopyramide, and lidocaine), and benzodiazepines (including diazepam, clorazepate, flurazepam, chlordiazepoxide, alprazolam, lorazepam, triazolam, midazolam, halazepam, prazezapam, and temazepam).

Various methods have been proposed for measuring concentrations of the commonly prescribed antidepressants in serum or plasma. These include chromatographic techniques such as quantitative thin-layer chromatography (TLC) (15), gas chromatography, and liquid chromatography. Gas chromatography has been used with flame ionization detection (15), nitrogen–phosphorus detection (16), electron-capture detection (15), and mass spectrometry (15, 17). Liquid-chromatographic procedures have been published for both normal-phase and reversed-phase columns (18–20). Various extraction procedures are also involved, varying from triple liquid–liquid extractions to solid-phase extractions. Color tests, such as Forrest's reagent, are also used by some laboratories to detect the presence of some of the tricyclics in toxicological specimens (21).

Immunoassays have also been developed for some of the antidepressants, especially the most commonly prescribed drugs: amitriptyline, nortriptyline, imipramine, and desip-
ramine (22-25). These immunoassays comprise radioimmunoassays, enzyme-multiplied immunoassay (EMIT), and fluorescent polarization immunoassay (TDx). There are immunoassays for both serum and urine; some are designed for therapeutic monitoring, others for toxicological screening.

Discussion

Ideally, the assay for any compound, including an antidepressant, would be fast, simple, inexpensive, specific, precise, accurate, and sensitive. The antidepressant class of drugs is used to treat other disorders, in addition to endogenous depression. Lower concentrations are typically necessary when the tricyclic is being used to treat chronic pain (26-28). Therefore, the sensitivity of the antidepressant assay to low concentrations of the drug and active metabolites is important.

Quantitative TLC is a tedious technique that only a few laboratories would consider suited for routine analysis. Instead, gas chromatography, liquid chromatography, and immunoassay techniques are the most commonly used methods for routine quantification of tricyclic antidepressants.

If gas chromatography is used, there is a choice of detection methods. Flame ionization has limited sensitivity, even with capillary columns, and therefore is of limited usefulness for determination of therapeutic concentrations, especially if the tricyclic is being prescribed, not for depression, but for migraine headaches or chronic pain, where lower doses typically are required. Flame ionization is successfully used, however, in screening for toxic concentrations of tricyclics in urine. Electron capture also is of limited usefulness, because derivatization is required for most of these tricyclics that do not contain a halogen atom in their structure. Only clomipramine and desmethylclomipramine have an intrinsic halogen. Nitrogen–phosphorus detection and mass–selective detection are the two methods of detection that are most useful for therapeutic monitoring of antidepressant concentrations in serum or plasma by gas chromatography.

Both normal-phase and reversed-phase liquid chromatography have proved useful for this, with ultraviolet light absorbance the most commonly used method of detection. Standard mobile phases as well as ion-pairing mobile phases have been used. These methods make it possible to resolve and quantify amitriptyline, nortriptyline, imipramine, desipramine, trimipramine, doxepin, desmethyldoxepin, protriptyline, and maprotiline in a single chromatographic injection. In addition, potentially cross-reactive compounds for the immunoassays, such as carbamazepine (Tegretol) and diphenhydramine (Benadryl), can also be separated from the tricyclics. Various chromatographic and immunochemical assays have a potential interaction with cyclobenzaprine (Flexeril) (29, 30). Cyclobenzaprine coelutes with either amitriptyline or imipramine in some gas chromatographic and liquid chromatographic systems. This interaction can be easily detected and eliminated from our liquid-chromatographic analysis by monitoring at two or more wavelengths (31). The cross reaction of cyclobenzaprine in immunoassays for antidepressants is more likely to occur in the presence of an overdose of cyclobenzaprine, when the concentration will be near the expected concentration of a tricyclic antidepressant.

Carbamazepine and diphenhydramine can significantly interfere with immunoassays, even though the relative cross-reactivity may be minor. For example, therapeutic carbamazepine concentrations are on the order of 50-fold the therapeutic tricyclic concentrations, and so even minor cross reactivity with carbamazepine would significantly affect results for a tricyclic antidepressant. Neuroleptics such as chlorpromazine and perphenazine also cross react with the immunoassays. This is a potential concern because many patients receive both an antidepressant and a neuroleptic. Neuroleptics rarely cause major interference problems, however, because the cross reactivity is minor and the concentrations are usually not high relative to the antidepressant.

Immunoassays for antidepressants have the same benefits as other immunoassays in clinical use. They are relatively simple and fast, requiring little or no sample preparation. But the disadvantages of the tricyclic immunoassays are the same as the disadvantages for any immunoassay. The radioimmunoassays involve radioactive waste disposal, particularly a problem for kits involving beta-emitting isotopes. All of the immunoassays have the potential for cross reactivity with other drugs, endogenous compounds, active metabolites, and metabolites with questionable or no activity. If the immunoassay does have good selectivity between the parent (amitriptyline or imipramine) and its predominantly active metabolite (nortriptyline or desipramine, respectively), then two separate immunoassays have to be performed when amitriptyline or imipramine are being measured. There is also the concern from cross reactivity of structurally similar compounds such as other antidepressants, cyclobenzaprine, carbamazepine, and diphenhydramine.

Some have wished for a radioreceptor assay for the antidepressants, similar to that used by some for the neuroleptics, but the potential usefulness of radioreceptor assays for antidepressants is very doubtful. First, there are several different types of receptor sites with probable activity for the antidepressants. Second, even if good models for receptor activity could be determined, we would still have the same concern that we currently have for radioreceptor activity assays for the neuroleptics: measurement of the receptor activity that is present in the serum or plasma may have little direct relationship to the activity of the drug at the active sites in the brain, because each drug will have a different relationship between the amount of drug present in the plasma or serum and the amount bound to receptors in the brain.

Conclusion

MEDTOX Laboratories is a reference toxicology laboratory. It provides emergency toxicologic screening, therapeutic drug monitoring for a wide range of drugs, forensic analysis, analysis for toxins from environmental or occupational exposure, and drugs-of-abuse screening for a national clientele. A complete array of instrumentation is used for immunoassay, atomic absorption spectrophotometry, liquid chromatography, and gas chromatography, including all methods of detection. At MEDTOX, we use a liquid chromatographic system consisting of an autosampler, liquid chromatograph with a normal-phase column, ultraviolet light detector, and computing integrator that is continuously dedicated to measurement of antidepressants for therapeutic monitoring. This system is available 24 hours a day for toxicological screening. We perform a solid-phase extraction and can generate a precise quantitative result within minutes.

We think that immunoassays may have a valuable role only in the initial toxicological screening for tricyclic-like
compounds. The immunoassays may also be very helpful in a laboratory that is dedicated to a limited patient group, especially if there are good controls over the number of prescribers per patient, the number of concomitantly administered antidepressant drugs, and perhaps the number of antidepressants that are on formulary and are available for clinical use, such that both the potential for assay interference and the requirement for multiple assays on a single specimen are minimized. Currently, however, about 5% of our specimens are positive for more than one antidepressant. Some patients may have prescriptions from two or more physicians, with none of the prescribers aware of the concomitant therapy. Approximately an additional 5% of specimens have the incorrect antidepressant assay ordered. The most common mis-order is when our laboratory is instructed to measure nortriptyline in a patient who is receiving desipramine (Norpramin). By our analysis we are able to report that nortriptyline is not present, but desipramine is present at the measured concentration. If that same analysis had been performed by immunoassay, the result would be either "none detected" or an erroneous quantitative result, depending on the selectivity of the particular immunoassay used. Another common ordering error is the request for assay of nortriptyline or desipramine when the patient is actually receiving amitriptyline or imipramine, respectively. The ratios of active metabolite to parent tricyclic can vary greatly. Therefore, it is not adequate to merely know the sum of the two, because the metabolite has greater pharmacological activity. Again, the type of reported error from an immunoassay would depend on the selectivity of the kit. In contrast, chromatographic analysis allows identification and correct reporting of all pertinent compounds.

One final concern regarding an assay that will quantify only one tricyclic at a time is "reverse metabolism." Some patients who are receiving nortriptyline or desipramine will show substantial concentrations of amitriptyline or imipramine, respectively. This reverse metabolism is frequently seen with overdoses of nortriptyline or desipramine.

Currently, very few laboratories offer analysis for additional metabolites of the tricyclics, such as the hydroxylated metabolites of desipramine and nortriptyline (32). Also, very few laboratories offer analysis for unbound tricyclics. But with more interest in the binding of tricyclics to plasma protein, an acute-phase reactive protein that varies widely in concentration, dependent on the patient's level of stress and inflammation (33), and the activity of these other metabolites, chromatographic assays capable of measuring metabolites and low concentrations of unbound drugs must be available in a reference toxicology laboratory. Sensitivity to low concentrations is also important to allow sampling 24–48 hours post-dose for predictive dosing protocols (34).

In the case of a potential overdose with tricyclics, the primary purpose for early toxicological analysis is to anticipate complications, such as hypotension and cardiac conduction disturbances, that might occur later in the patient's clinical course (35, 36). These drugs have markedly different potencies on muscarinic and pre-synaptic α receptors, leading to toxicities of varying degrees (1, 37–39). Emergency-room physicians need to know this information to evaluate the potential seriousness of an ingestion and determine whether the patient should be hospitalized or released. The medical team and cardiologists who are following the overdosed patient one to three days post-admission need to know what degree of toxicities to expect from the drug used and the specific concentrations. Initial hemodynamic and electrocardiographic monitoring are not totally predictive of how intensively the patient should continue to be monitored. The delayed toxicities of the antidepressants are unique and should never be minimized. The concentration of the specific antidepressant helps determine how aggressively the patient needs to be monitored for future complications. Assume, as an example, a patient who on admission has measurable concentrations of doxepin, 600 ng/mL, and desmethyl doxepin, 200 ng/mL. The total tricyclic concentration is 800 ng/mL. The Abbott TDx assay, however, would read approximately 300 ng/mL because of the cross-reactivity of the antibody. Does that result accurately reflect the patient's clinical condition? No toxicologist should be satisfied with knowing a cross-reactive sum of tricyclic-like compounds without knowing the components and therefore the true drugs and concentrations present. The immunoassay should not stand as the sole test for antidepressants even in the case of an overdosed patient. There are too many such potential, and possibly yet unknown, interactions for the laboratory to routinely rely only on immunoassays for therapeutic drug monitoring or toxicological identification of antidepressants.

References