Resolving Erroneous Reports in Toxicology and Therapeutic Drug Monitoring

A Comprehensive Guide

Amitava Dasgupta

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Preface

Therapeutic drug monitoring and testing for drugs of abuse are important clinical laboratory tests that have a significant impact on patient safety and patient management. Physicians rely on serum or whole blood levels of a therapeutic drug for dosage adjustment and also to interpret significant drug–drug interactions. Therefore a falsely elevated or falsely lowered drug concentration due to the presence of an interfering substance in the specimen has a very serious impact on patient safety. The interference can be a false positive or a false negative. A clinician usually questions the validity of a test result if the concentration of a therapeutic drug is unexpectedly high. However, negative interference has more serious clinical consequences because it occurs infrequently compared with positive interference, and a clinician may simply increase the dosage of the medication without recognizing that the drug concentration may be falsely low due to the presence of an interfering substance in the specimen. There are reports in the literature of severe digoxin toxicity due to increased digoxin dosage based on reports of low digoxin concentration due to negative interference (see Chapter 12). Drugs of abuse testing using immunoassays is subjected to interference. Many over-the-counter cold and cough medications containing ephedrine or pseudoephedrine may cause a false-positive amphetamine immunoassay test result due to cross-reactivity with antibodies used in these immunoassays. A false-positive phencyclidine test result due to the presence of dextromethorphan, a common ingredient in many over-the-counter cold medications, is well documented in the literature. Such false-positive test results are not of concern for workplace drug testing because positive immunoassay

test results are always confirmed by a chromatographic method, most commonly gas chromatography combined with mass spectrometry (GC/MS). However, for medical drug testing where GC/MS confirmation is not available, a physician may falsely accuse a patient regarding his or her drug abuse based on a false-positive immunoassay result, although in reality the patient is not a drug abuser. This may hamper physician–patient trust or may cause mental agony to the patient. A false-positive blood alcohol result using an enzymatic alcohol assay has a similar consequence. A false-positive blood alcohol level measured by breath analyzer may have a serious legal impact because a driver may be falsely accused of driving under the influence of alcohol (see Chapter 7 for a detail discussion on this topic).

This book provides a comprehensive guide for laboratory professionals and clinicians regarding the sources of errors in therapeutic drug monitoring and drugs of abuse testing and how to resolve such errors and identify discordant specimens. Error-free laboratory results are essential for patient safety. Because herbal medicines are widely used by the general population, drug–herb interactions are discussed. For example, warfarin is known to interact pharmacokinetically and pharmacodynamically with many drugs; Chapter 9 discusses many clinically significant interactions of warfarin with herbal supplements. Chapter 16 is devoted to other important drug–herb interactions where an apparent erroneous result in therapeutic drug monitoring is due not to the presence of an interfering substance but to clinically important drug–herb interactions. Clinical laboratory testing is also helpful in the diagnosis of certain plant poisoning and toxicities from the use of certain herbal supplements (Chapter 10).

This book is intended as a practical guide for laboratory professionals and clinicians who deal regularly with erroneous results in therapeutic drug monitoring and drugs

of abuse testing. I hope this book will help them become more aware of such sources of errors and empower them to eliminate such errors when feasible.

I would like to thank Robert L. Hunter, chairman of the Pathology and Laboratory Medicine Department at the University of Texas–Houston Medical School, for his support when I worked on the project. I also thank Alice Wells for critically reading the entire manuscript and making helpful suggestions. Last but not least I thank my wife, Alice, for tolerating my long hours spent on writing the book on weekdays and weekends. Finally, readers will be the judge of the final success of this book. If they find this book useful, that will be my best reward for writing it.

Amitava Dasgupta
Houston, Texas

Chapter 1

An Introduction to Tests Performed in Toxicology Laboratories

1.1. INTRODUCTION

In general therapeutic drug monitoring, urine toxicology drug screens, analysis of blood alcohol and volatiles as well as emergency toxicology drug screenings are commonly offered tests in a toxicology laboratory. Certain drugs with a narrow therapeutic range require routine monitoring, and in general serum or plasma is the preferred specimen. However, certain immunosuppressant drugs such as cyclosporine, tacrolimus, sirolimus, and everolimus are monitored in whole blood, although another immunosuppressant, mycophenolic acid, is monitored in serum or plasma. Drug screening for a patient with a suspected drug overdose is more commonly performed using urine specimens, but blood and gastric fluid specimens are also analyzed for the screening of drugs in case of a suspected recent overdose. In addition, blood alcohol analysis is also commonly conducted in toxicology laboratories because alcohol use alone may cause life-threatening intoxication. In addition, many abusers of illicit drugs also consume alcohol at the same time to achieve euphoria. Bogstrand et al reported that psychoactive substances were found in approximately 50% of the patients admitted to the hospital within 12 hours of injury. Of a total

of 1272 patients studied (510 women and 762 men), 38% of the women and 48% of the men had a positive blood sample of a psychoactive drug on admission. Alcohol was the most prevalent substance; 27% of patients had a positive blood alcohol test. Cannabis was the most prevalent illicit drug (6.2%); diazepam was the most common drug, detected in 7.4% of patients. The authors concluded that alcohol was the most common substance found in these patients and was particularly related to violence, whereas medicinal drugs were most prevalent in accidents at home (1). Alcohol is also a risk factor for injury in adolescents. Injured adolescents are more likely to visit the emergency department with an alcohol-related event during the early hours of the morning (2). Multiple abused drugs are also encountered in severely intoxicated patients and individuals who die from a drug overdose. Dickson et al reported a case of a 22-year-old white man who died from a drug overdose. Routine toxicological analysis detected morphine in the decedent's blood (0.06 mg/mL). In his urine specimen, 6-monoacetyl morphine (a marker compound for heroin abuse), morphine, codeine, doxylamine, and mephedrone were confirmed (3). In addition to poisoning due to alcohol, an overdose with various drugs may provoke a visit to the emergency department. Both salicylate and acetaminophen are commonly encountered drug in poisoned patients, and such drug levels are often screened in a toxicology laboratory using serum or plasma specimens.

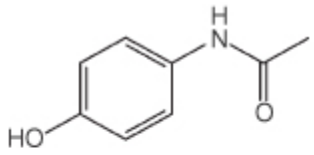
1.2. ACETAMINOPHEN AND SALICYLATE ASSAYS

Acetaminophen (paracetamol) overdose, both intentional and accidental, remains a significant public health concern. In one report, the authors calculated that from 2000 to

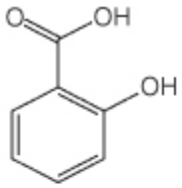
2006, an age-adjusted rate of hospitalization related to acetaminophen was 13.9 per 100,000 population in the United States. Most acetaminophen overdoses were intentional (4). Acetaminophen can also cause liver toxicity. Because acetaminophen is a component of many medications, both prescription and over the counter, unintentional overdose can occur. Concurrent use of alcohol may also potentiate hepatoxicity of acetaminophen (5). Chronic alcohol abusers are also at an increased risk of acetaminophen-induced hepatotoxicity even after therapeutic use (6).

Salicylate poisoning is also common, and an adult can die from it. In 2005, according to the Toxic Exposure Survey from the American Association of Poison Control Center's National Poisoning and Exposure Database, there were more than 20,000 reported exposures from salicylate, and 64% of these patients were treated in a health care facility. It was considered that 50% of all exposures were intentional, and 60 patients died from a salicylate overdose (7). Galbois et al reported the case of a 74-year-old schizophrenic patient who died of salicylate poisoning; his blood salicylate level was 876 mg/L (87.6 mg/dL, a very toxic level) (8). Salsalate is a nonsteroidal anti-inflammatory drug that is mostly metabolized to two molecules of salicylic acid. However, approximately 7–10% of the drug is not hydrolyzed to salicylic acid and can be recovered in the urine either as the unchanged drug or as glucuronide conjugate (9). Delayed salicylate toxicity without early manifestation may occur after overdose with both salicylate and salsalate. Chemical structures of salicylate, salsalate, and acetaminophen are given in Figure 1.1.

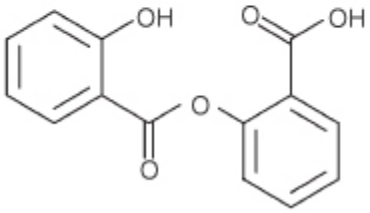
Figure 1.1. Chemical structures of acetaminophen, salicylate, and salsalate.



Acetaminophen



Salicylate



Salsalate

Case Study

A 14-year-old girl ingested 120 tablets of 81 mg aspirin extended release and 6 tablets of ciprofloxacin 2 hours prior to arrival at the emergency department. Upon arrival she denied nausea, shortness of breath, diaphoresis, or abdominal pain. Activated charcoal 50 g with sorbitol was administered orally for decontamination. No acetaminophen or ethanol was detected in her blood. In addition, a urine drug of abuse screen was also negative. The first salicylate blood level was 1 mg/dL (therapeutic: 10–20 mg/dL) drawn 4 hours after ingestion, but the salicylate level was elevated to 13 mg/dL 6 hours after ingestion, and the patient remained asymptomatic. The patient remained asymptomatic until 35 hours after exposure when she developed dizziness, tinnitus, and epigastric discomfort, and her blood salicylate concentration was elevated to 46 mg/dL. A second dose of 50 g of activated charcoal was administered along with bicarbonate infusion. She did not develop any renal failure, and after an observation period she was discharged to a psychiatric facility. The cause of delayed salicylate toxicity was unclear. Possibilities include delayed absorption due to enteric-coated or extended-release formulation, or the formation of bezoars (aggregates of drug that form a soft mass with limited surface area exposed to gastric fluid). The interior portion of the drug mass has mostly undissolved drugs. Other than salicylate, a variety of medications may form such bezoars (10).

Case Study

A 31-year-old man with a history of depression, posttraumatic stress disorder, and prior attempted suicide was discovered by his neighbor in the morning with greatly reduced consciousness. He was transferred to a hospital by the emergency medical team, and he admitted that he had attempted suicide the previous night by overdose but did not disclose the medication taken. Thirty-six new and old pill bottles were found on the scene that included acetaminophen, hydrocodone with acetaminophen, hydroxyzine, ibuprofen, lorazepam, magnesium oxide, morphine, oxycodone, paroxetine, ranitidine, salsalate, temazepam, tramadol, venlafaxine, and zolpidem. On arrival at the hospital, his blood pressure was 162/92 mm Hg, pulse 100 bpm, respiratory rate 14/min, and 98% oxygen saturation at room air. Toxicological investigation revealed a serum salicylate level of 29.2 mg/dL, and a urine drug screen was positive for benzodiazepines and cannabinoids. After 3 hours, the patient's level of consciousness and respiratory rate both decreased, and an arterial blood gas showed a pH of 7.31, a pCO_2 of 48, and pO_2 of 111. He was intubated 5.5 hours after admission due to apnea, and sodium bicarbonate was administered intravenously. In addition, two doses of activated charcoal (50-g dose) were administered by nasogastric tube. Salicylate concentration then peaked to 55 mg/dL just over 8 hours after presentation to the emergency department. Later his salicylate blood level declined, and sodium bicarbonate therapy was discontinued. Unfortunately, his blood salicylate level increased again later, peaking at 61.7 mg/dL 67 hours after presentation. The patient was later extubated and kept on a psychiatric hold with a one-to-one sitter. Salicylate ingestions are known to demonstrate unusual toxicokinetics and absorption patterns during overdose, and in this case a return to a toxic salicylate level was observed after apparent resolution of toxicity (11).

Acetaminophen and salicylate in serum, plasma, or urine can be measured by commercially available assays that may be either based on colorimetric principle or are immunoassays. These assays can be run on various automated analyzers. In addition, chromatographic methods such as high-performance liquid chromatography or gas chromatography can also be used for the determination of both acetaminophen and salicylate in various biological matrixes. Gaspari and Locatelli described a simple high-performance liquid chromatographic determination of both salicylate and acetaminophen in plasma after liquid-liquid extraction with hexane and ultraviolet detection at 228 nm

(12). Miceli et al also described a liquid chromatographic method for the determination of salicylate and acetaminophen in human plasma using 8-chlorotheophylline as the internal standard (13). However, chromatographic procedures are labor intensive, and in toxicology laboratories, various automated assays are commonly used for routine determination of both salicylate and acetaminophen. Unfortunately, these automated assays are subjected to interferences, and the presence of high bilirubin in serum or plasma may affect both the acetaminophen and salicylate assays. Stewart and Watson reviewed various methods available for the estimation of salicylate and acetaminophen in serum, plasma, and urine (14).

A false-positive acetaminophen level due to hyperbilirubinemia has been reported. In one report, the authors observed false-positive acetaminophen levels in two patients who had high bilirubin concentrations (25.5 mg/dL and 40.1 mg/dL, respectively) in their sera using the GDS Diagnostics enzymatic acetaminophen assay (GDS Diagnostics, Elkhart, IN). However, enzyme-multiplied immunoassay technique (EMIT) (Syva, Palo Alto, CA), acetaminophen assay, and gas chromatography/ mass spectrometric (GC/MS) assay did not reveal the presence of acetaminophen. The GDS assay utilizes an enzyme (n-arylacylamidase) to convert acetaminophen into para-aminophenol and acetate. Then p-aminophenol reacts with ortho-cresol in the presence of periodate to form the chromophore indophenol, which has a strong absorption spectra at 615 nm. The EMIT assay utilizes an antibody that recognizes acetaminophen. Although the mechanism of interference with the GDS enzymatic assay is unknown, the authors speculated that bilirubin may form a complex with ortho-cresol (15). Polson et al concluded that false-positive acetaminophen test results may occur when bilirubin

concentration is above 10 mg/dL, leading to potential clinical errors especially with enzymatic-colorimetric assays (16). Significant positive bias of bilirubin in the Trinder reaction-based salicylate methods (color complex formed due to reaction of salicylate with ferric ions) on automated analyzers has been reported. However, such interference can be eliminated by using the fluorescence polarization immunoassay for salicylate using the AxSYM analyzer (Abbott Laboratories, Abbott Park, IL) (17). Broughton et al also described interference of bilirubin on a salicylate assay performed using the Olympus automated analyzer (18). Mitochondrial acetoacetyl-CoA thiolase deficiency is a rare metabolic disorder causing acute episodes of severe ketosis and acidosis. Tilbrook reported false-positive salicylate in an 18-month-old boy who presented to the hospital with severe acidosis. The authors concluded that false-positive salicylate using the Trinder reagent was due to the interference of a high level of acetoacetate in the specimen that interfered with the assay (19). However, immunoassays for salicylate manufactured by various diagnostic companies are free from such interferences.

Case Study

A 31-year-old woman was admitted to the hospital for abdominal pain, decreased appetite, malaise, confusion, and tea-colored urine. Investigation showed acute liver failure characterized by high bilirubin (70.7 mg/dL), alanine aminotransferase 6170 U/L, aspartate aminotransferase 5080 U/L, lactate dehydrogenase 6830 U/L, and alkaline phosphatase 150 U/L. Plasma acetaminophen concentration of 121 µmol/L (therapeutic up to 100 µmol/L) resulted in suspicion of an acetaminophen overdose as the probable cause of liver failure because serological tests for hepatitis A and B were negative. However, her plasma acetaminophen level remained elevated even on day 3 (104 µmol/L) raising the suspicion of bilirubin interference in acetaminophen measurement because the acetaminophen assay on the Vitros analyzer (Johnson & Johnson, Rochester, NY) is based on the enzymatic conversion of acetaminophen to para-aminophenol and subsequent reaction with ortho-cresol to form the blue-colored complex indophenol, which is measured by change in absorption at 600 nm. High bilirubin interferes with the assay. When the authors measured acetaminophen concentration using protein-free ultrafiltrate, which is free from protein-bound bilirubin, the acetaminophen concentration was below the detection limit of the assay, indicating no acetaminophen was present in the plasma. When acetaminophen was remeasured using a chromatographic method, no acetaminophen level was detected, further establishing that the initial high acetaminophen result was a false-positive result due to the interference of bilirubin with the acetaminophen assay (20).

1.3. ANALYSIS OF ALCOHOL

Alcohol is a major cause of motor vehicle accidents, and such victims are treated in the emergency department of hospitals. Blood alcohol testing is a routine and widely ordered test in a toxicology laboratory. Blood alcohol can be measured by either an enzymatic method or by gas chromatography. Although enzymatic methods can be automated and are often applied for measuring blood alcohol in busy toxicology laboratories, these methods also suffer from interferences, especially if both high lactate and

lactate dehydrogenase are present in the specimen. However, gas chromatographic methods are free from such interferences, and such methods should be used for legal alcohol determination. This important topic is discussed in detail in Chapter 7. In addition, gas chromatographic methods are capable of analyzing other volatile compounds such as methanol, isopropyl alcohol, acetone, ethylene glycol, and related volatile compounds along with alcohol (ethyl alcohol) simultaneously.

1.4. THERAPEUTIC DRUG MONITORING

The International Association for Therapeutic Drug Monitoring and Clinical Toxicology adopted the following statement to describe therapeutic drug monitoring, “Therapeutic drug monitoring is defined as the measurement made in the laboratory of a parameter that, with appropriate interpretation, will directly influence prescribing procedures. Commonly, the measurement is in a biological matrix of a prescribed xenobiotic, but it may also be of an endogenous compound prescribed as a replacement therapy in an individual who is physiologically or pathologically deficient in that compound” (21). Therapeutic drug monitoring has been used in clinical practice since the 1970s with the goal of personalizing the dosage of a drug for maximum efficacy and minimal toxicity. Usually therapeutic drug monitoring is necessary for a drug with a narrow therapeutic window, and only a small fraction of all drugs available require therapeutic drug monitoring. In general, therapeutic drug monitoring is not needed for any over-the-counter drug because these drugs usually have a wider margin of safety. However, the intentional or accidental overdose of over-the counter medications such as

salicylate or acetaminophen is troublesome because such an overdose may even be fatal.

A drug may be administered to a patient via various routes including oral, rectal, intravenous, intramuscular, transdermal, or through sublingual application. Each route of administration has its advantages and disadvantages. For example, the oral route of administration is easiest for a patient, but the drug may suffer low bioavailability due to first-pass metabolism or intake of food or the bioavailability may be higher if the patient consumes alcohol. Moreover, a peak drug level may be achieved after a long delay. In contrast, peak concentration can be achieved rapidly if the drug is administered intravenously or intramuscularly, but that route of administration may result in patient discomfort. Rapid absorption of a drug can be achieved by sublingual application, but the drug may undergo first-pass metabolism thus reducing the efficacy of the drug. Usually a drug is poorly absorbed after transdermal application, and absorption may also be low after rectal application of a drug. In addition, most drugs that require therapeutic drug monitoring are delivered orally except for vancomycin and aminoglycoside. Criteria for drugs to be a candidate for therapeutic drug monitoring are the following:

1. Narrow therapeutic range where the dose of a drug that produces the desired therapeutic concentrations is also closer to the dose that may also cause toxic serum concentration. Serious toxicity may be encountered if the drug is not monitored.

2. There is an unpredictable relationship between dose and clinical outcome but a predictable relation between serum or whole blood drug level and clinical efficacy as well as toxicity. Significant changes in metabolism due to genetic makeup, age, sex, or disease for these drugs are responsible for the poor relation between the dosage and the drug level in the blood.

3. Drugs that demonstrate nonlinear pharmacokinetic parameters are also candidates for therapeutic drug monitoring.

4. Toxicity of a drug may lead to hospitalization, irreversible organ damage, and even death; for example, vancomycin may cause irreversible ototoxicity.

1.4.1. Drugs Requiring Therapeutic Drug Monitoring

Most drugs monitored in clinical laboratories are administered to patients with chronic diseases. These drugs are often used as a prophylactic agent to prevent reoccurrence of symptoms. For example, phenytoin is used to prevent certain types of convulsions in patients. Patient compliance is a major issue for successful drug therapy, and often patients do not take drugs as recommended, especially when they are dealing with a chronic illness. Gillisen reported that in patients with asthma, the adherence rates to medications are sometimes below 50% (22). Patsalos et al concluded that therapeutic drug monitoring of anticonvulsant drugs is beneficial to assess compliance especially in patients with uncontrolled seizures and breakthrough seizures (23). The cure rate for acute lymphoblastic leukemia (ALL) may exceed 85%, but up to 3 years of maintenance therapy with weekly methotrexate and daily 6-mercaptopurine is needed. Therefore, compliance with therapy is essential for the cure of ALL. In one report, the authors compared direct structured interview, the search of lack of compliance documented in the clinical record, and therapeutic drug monitoring of methotrexate to investigate compliance with therapy among children receiving such treatment. In 5 of 49 interviews, at least an episode of noncompliance was observed; searching clinical records revealed 8 of 49 patients skipped taking