Ashraf Mozayani · Lionel Raymon
Editors

Handbook of Drug Interactions

A Clinical and Forensic Guide

Second Edition

Humana Press

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Preface

Adverse drug reactions and drug interactions remain a major issue in 2011. During the second edition of our book, FDA reported greater than 370,000 serious adverse events in 2009 and more than 100,000 for the first quarter of 2010. The Adverse Event Reporting System is a database that gives computerized statistics used to support FDA's post-marketing safety surveillance for all approved drugs. A serious event is defined as requiring hospitalization, being life-threatening, causing disability or congenital anomalies, for example. Importantly, more than 63,000 deaths were recorded in 2009, and more than 20,000 occured during the first quarter of 2010.

The second edition of *Handbook of Drug Interactions: A Clinical and Forensic Guide* has been updated to reflect new information and also includes new chapters of interest. In this respect, it is a continuation of the first edition and part of the ongoing story of drug–drug interactions.

Pharmacogenomics is a rapidly growing field covering the genetic basis for individual variability in drug responses. This new section allows the reader to review important polymorphisms in drug metabolizing enzymes and applies the findings to forensic interpretation through interesting cases involving opiates.

Although the section relating to central nervous system drugs encompasses a number of potential drugs with illicit use such as benzodiazepines and opiates, a chapter dealing exclusively with drugs of abuse has been added to the second edition. Cocaine, amphetamines, cannabis, flunitrazepam and GHB are now discussed. Alcohol and nicotine are still covered in the section related to environmental and social pharmacology.

The existing chapters from the first edition have, in most cases, been updated and edited to reflect new data or bring out better tables and diagrams. More recent drugs and formulations are included. Recent references have been added for completeness.

This volume emphasizes explanations when possible and covers both pharmacokinetic and pharmacodynamic drug interactions. The result, we hope, will continue to prove useful to health and forensic professionals as well as students.

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Part I
Pharmacogenomics

Chapter 1
Pharmacogenetics in Clinical and Forensic Toxicology: Opioid Overdoses and Deaths

Saeed A. Jortani, Elaine Stauble, and Steven H. Wong

Abstract Factors considered in the observed variability in drug response within a population are intrinsic, extrinsic, or a combination of both. The intrinsic factors are differences in the demographics of a given individual (e.g., age or gender), disease or physical condition (e.g., renal function or BMI), and pharmacogenetics (see below). The extrinsic factors are composed of environmental factors (e.g., diet) as well as drug interactions or polypharmacy.

In recent years, the role of genetic variation in drug metabolism and response has been increasingly recognized. Since various pharmacokinetic and pharmacodynamic mediators of drug efficacy and toxicity involve peptides and proteins, polymorphisms in the genes responsible for encoding their amino acid sequence create a fundamental mechanism for the observed variations. In this chapter, we will briefly discuss the sources of variability in drug metabolism and response. The role of pharmacogenetics in pharmacokinetics and pharmacodynamics will then be discussed. Special attention will be paid to the consequence of polymorphisms on the forensic applications of toxicology, such as postmortem investigations.

Keywords Pharmacogenetics • Variability • Polymorphisms • Forensic applications

Pharmacogenetics and Pharmacogenomics

The terms pharmacogenomics and pharmacogenetics are generally used interchangeably, denoting the study of genetic variation on an individual's ability to metabolize a drug or respond to it. More specifically, pharmacogenetics is concerned with the effects of variation in one or a handful of genes whereas pharmacogenomics

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Table 1.1 Drugs used in pain management as analgesics or as adjutants

Drug class	Examples
Analgesics	
NSAIDs	
Traditional	Aspirin, ibuprofen
Coxibs	Celecoxib, rofecoxib
Opioids	
Strong opioids	Fentanyl, morphine, hydromorphone
Partial agonists	Buprenorphine, pentazocine
Weak opioids	Codeine, hydrocodone, propoxyphene
Local anesthetics	Lidocaine
Neuroleptics	Phenothiazines, clozapine
Tricyclic antidepressants	Nortriptyline, despiramine
SSRIs	Lamotrigine, citalopram, sertraline
Antiepileptics	Barbiturates, carbamazepine
NMDA antagonists	Ketamine, methadone[a]

SSRIs selective serotonin reuptake inhibitors

[a]Methadone and tramadol elicit their pharmacological actions through opioid receptors and by an additional mechanism such as NMDA antagonism or inhibition of reuptake of norepinephrine and serotonin

involves the entire genome [1]. The field of clinical pharmacogenetics was initiated approximately a decade ago [2, 3] with a slow but steady adaptation in various fields of medicine such as oncology [4], psychiatry [5, 6], and cardiology [7–9]. In fact, the role of pharmacogenetics in warfarin management has led to clinical testing for polymorphisms in Cytochrome P450 2C9 (CYP2C9) and Vitamin K Oxido-Reductase Complex 1 (VKOR C1) genes [10–12]. This has also involved the development of several clinical decision tools that now make it possible for clinicians to incorporate genotyping results in their decisions regarding warfarin therapy [13, 14]. Such progress has led to recommendations by regulators and guidelines by various authoritative bodies [10, 15–17]. The significant role of pharmacogenetics in oncology has also been noticeable involving multiple drugs such as Erbitux (cetuximab) and K-Ras mutation [18], tamoxifen and CYP2D6 testing [19], and Irinotecan and UGT1A1 testing [20, 21]. In pain management, pharmacogenetics has been implicated for various non-steroidal anti-inflammatory drugs (NSAIDs) such as Celecoxib [22] and opioids such as fentanyl, hydrocodone, and codeine [23–27]. Table 1.1 lists various classes of drugs used either directly or as adjuvants in pain management [28, 29]. Opioids constitute a major class of analgesics with many of the members being influenced by pharmacogenetic variables. Codeine, hydrocodone, and oxycodone are substrates for CYP2D6 whereas the pharmacokinetics of buprenorphine and fentanyl are influenced by CYP3A4 and CYP3A5 enzymes [30, 31]. Oftentimes, the same enzymes are responsible for the metabolism of additional drugs also given to the patients for various reasons. Our discussion in this chapter will demonstrate the use of pharmacogenetics for forensic applications focusing primarily on opioids. Through the review of several cases, we

will highlight the importance of considering genetic variations in interpretation of postmortem drug concentrations in the field of forensic toxicology. Obviously, there is a steep learning curve for general toxicologists and pharmacologists trying to bring genetic information into their applied practices. Wong and colleagues first coined the term molecular autopsy, which best signifies the role of incorporating pharmacogenetics in forensic toxicology [25]. It is our hope that this chapter will catalyze the adaptability of this novel approach to describe the mechanistic role of pharmacogenetics in personalized medicine as well as in personalized justice. This latter emerging practice would include the use of molecular diagnostics such as pharmacogenomics in legal proceeding to explain the possible genetic contribution to drug therapy and efficacy, and therefore performance and side effect. This might be applied in the settings of drug influence of drugs (DUID) and working under the influence of drugs (WUID). According to Wong, the inevitable check and social balance relationship to personalized medicine would enhance both practices in the future [70, 71]).

Variability in Response to Medications

Forensic toxicologists are among the professionals facing the interpretive challenges brought about by variability in drug response and efficacy. Frequently, such variabilities are co-presented in settings affected by additional confounders such as postmortem redistribution, polypharmacy, unknown drug exposures, and homicidal or suicidal poisonings. In this section, we will briefly discuss physician variability and genetic differences in drug handling and response that are considered two of the main factors affecting interpretation of clinical and forensic toxicology results.

Physician Variability

To demonstrate the issue of physician variability, we will focus on the use of medications in the area of pain management. Differences among practitioners in this medical discipline have led to either inadequate pain management for patients, accusation of drug diversion or non-compliance, as well as considerable morbidity and death. Various tools such as drug screening, patient contracts, and counseling have been developed to cope with these challenges.

Much attention in the lay press, as well as the medical literature, has focused on pain control in the last several years. In emergency rooms, only 44% of patients rate their pain control as “very good” [32]. This is especially interesting in light of the fact that after Lipitor, hydrocodone (Lortab) is the second most commonly dispensed prescription medication in this country [33]. What are the factors that influence clinical decision-making on the part of physicians prescribing opioid narcotics? The decision to prescribe narcotics is quite complex. It varies depending on the characteristics of the physician and the presenting condition, as well as patient characteristics.

There is a large body of research focusing on physician variables, as well as on the different clinical conditions with which patients present. Studies reveal that there is an inherent dichotomy between beneficence of the physician versus the physician who acts as gatekeeper to forestall narcotic addiction. Every physician approaches a problem from his or her own perspective. The decision to prescribe opioids depends on the physician's personal experience (i.e., cultural, surgical). It must depend on the clinical content of the situation (i.e., the chief complaint, their experience investigating the chief complaint, stereotyping), as well as the context (role expectation, available resources). Patient expectations and demands also affect the decision to prescribe narcotics. Some physicians prescribe more, others less, when the patient requests "something strong" for the pain. The effectiveness of the communication between the patient and doctor also plays a role. Language barriers make the physician–patient interaction cumbersome; interpreters for a specific dialect are not always readily available. Male and female medical students have been shown to respond differently to identical clinical vignettes depicting chest pain [34]. Their responses also varied depending on the patient's race and gender. Each physician's training and philosophy of prescribing narcotics develops depending on what medical school they attended, how long ago they graduated, and their surgical experience. The specialty of the physician (i.e., ER physician versus general practitioner) also influences the prescribing of opioid narcotics. General practitioners may respond differently to patients with chronic non-cancer pain than the ER physician, who is accustomed to treating acute pain. The general practitioner often has more continuity with the patient, knows their family history in depth, and has more information with which to make a decision regarding prescriptions. In contrast, the ER physician makes decisions in a vacuum, relatively speaking. This may permit judgmental issues to be more influential, especially at the beginning of an encounter with a patient for whom the physician has a paucity of objective data. When ER physicians were faced with clinical scenarios of three common medical conditions in a study designed by Tamayo-Sarver et al. [35], patient race and ethnicity had no effect on whether the physician prescribed narcotics or not. When information about high socioeconomic status or socially desirable occupations was provided with the same scenario, the physician prescribed more opioid narcotics. In another series of cases from a pain clinic, the severity and duration of the pain experienced by the patient did not affect narcotic prescribing as much as observed pain behaviors (distorted posture, audible expressions of distress, and avoidance of activity) [36]. The communication skills possessed by the clinician have a large influence on his/her decision to prescribe medication for pain control. Physicians look for features compatible with their expectation about a specific clinical condition [35]. When ER physicians viewed identical case scenarios, they had highly variable rates of prescribing narcotics. Physician prejudice and stereotyping also plays a role and occasionally may threaten the patient–physician relationship.

Therefore, the complexity of a clinical decision to prescribe opioid narcotics for pain control is apparent. It may be that better curricula must be developed early on in medical schools, to standardize the prescribing of opioids for certain clinical

conditions, so as to "level the playing field," and to better control pain for all patients with the same condition, no matter how differently they present.

In summary, from a physician's standpoint, effective pain management is complicated by multiple factors, including strict regulatory requirements and concerns about addiction or diversion, and also because both the experience and treatment of pain are subject to a broad degree of interindividual variability. Setting policy and procedural issues aside, the very subjective nature of pain is at the heart of the problem for practitioners. Research has found that the experience of pain and patients' response to therapy (with regard to adverse reactions and therapeutic benefit), are subject to wide interindividual variability caused by a number of factors, including patient age, BMI, organ function, co-medication, underlying disease, and genetics. In the remainder of this chapter, we will focus on the genetic variability influencing toxicology and interpretation of drug response.

Genetic Differences in Drug Handling and Response

The effect of physician variability is theoretically minimized by a scenario in which the same clinician is prescribing a given medication for two different patients. An example is pain medication administered to these two individuals with similar extrinsic factors. It is widely recognized that even under these circumstances, variability in response remains unlikely. Since proteins and peptides are responsible for the action of therapeutics, alterations in the genetic sequence responsible for encoding them creates an inherent source of variability. The association between drug response and toxicity and inherited genetic variations was recognized over 50 years ago [37]. Several different types of variations exist in the DNA sequence which range from single nucleotide polymorphisms (SNPs) to larger structural alterations such as copy number variants (CNVs), deletions, and inversions [38, 39]. Polymorphisms are defined as genetic variants occurring in at least 1% of the population. By the year 2007, over 3.2 million SNPs in the human genome have been reported [40]. The functional consequences of SNPs range from having no effect on the transcribed protein's function to a total loss of its activity. Since SNPs can alter a drug's pharmacokinetics and pharmacodynamics, they serve as an objective measure of a potentially significant source of variability in drug response. In fact, clinical pharmacogenetics has now made it possible for incorporating the effect of such variability in dosing decision-making and personalized drug therapy [20].

Polymorphisms in Drug Metabolizing Enzymes

A significant part of genetically caused variations in drug handling arise from the mediators of pharmacokinetics such as the drug metabolizing enzymes. These enzymes are classified into two main groups based on their function as phase

I–oxidative or phase II–conjugative [41]. In the clinical pharmacogenetic practice, many of the phase I and phase II enzymes are currently genotyped for assessing an individual's variability in drug metabolism. Within this group, CYP450 and several phase II enzymes such as urindine diphosphate glucuronosyltransferase 1A1 (UGT1A1) take part in the metabolism of the majority of drugs approved in the USA. Zanger et al. have studied the elimination routes for the 200 drugs available mainly by prescription in the USA [31]. Approximately 80% of drugs for which hepatic metabolism is indicated, polymorphisms in CYP450 genes of the families 1, 2, and 3 are considered to be the main sources of variability. Contribution by CYP3A4/5 was shown to be responsible for metabolism of 37% of the drugs studied. The extent of involvement by other CYP enzymes was reported to be 17% for CYP2C9, 15% for CYP2D6, 10% for CYP2C19, 9% for CYP1A2, 6% for CYP2C8, and 4% for CYP2B6 [31]. The Food and Drug Administration (FDA) has long recognized the importance of incorporating pharmacogenetic knowledge and testing in clinical practice. The FDA has made significant efforts in relabeling products where drug efficacy or toxicity has been linked to polymorphisms (Wu et al. Future medicine 2009). Genotyping tests for several enzymes, including CYP2D6, CYP2C9, CYP2C19, and UGT1A1 as well as a drug target (i.e., VKORC1), have been approved by the FDA as clinical laboratory tests. There are many articles and book chapters devoted to presenting pharmacogenetics of various classes of drugs and genes for clinical applications [8, 14, 16]. Since covering all of these is beyond the scope of this chapter, we will focus on the CYP2D6 and opioid analgesics in the setting of pain management and the associated forensic cases. Special attention will be paid on drugs more likely to be implicated in postmortem cases and issues related to forensic toxicology.

CYP3A4 and CYP3A5

The CYP3A subfamilies are overall the most abundant drug metabolizing enzymes, taking part in the metabolism of approximately 40% of the drugs [31]. In this subfamily of enzymes, the CYP3A4 and CYP3A5 are the two most important ones in the hepatic tissue. Many drugs of interest to forensic toxicologists are the semisynthetic or synthetic opioids which are either in part or primarily metabolized by the CYP3A4 enzyme. These include methadone, propoxyphene, buprenorphine, tramadol, and fentanyl [42, 43]. Generation of norfentanyl from fentanyl by CYP3A4 has previously been reported in several forensic cases [25]. Another example of variability is N-dealkylation of buprenorphine to norbupronorphine [43] by CYP3A4 [44]. Although buprenorphine has low respiratory depressive properties, its metabolite is the one that primarily contributes to its toxicity [43, 45]. Another issue to be considered by toxicologists while interpreting drug levels is the coadministration of opioid analgesics with drugs known to alter the activities of CYPA4/5 enzymes. To demonstrate this point, consider taking itraconazole or ketoconazole and even drinking grapefruit juice which are all known to inhibit the CYP3A4 activity in patients also

on fentanyl or buprenorphine. These inhibitors are expected to enhance fentanyl's toxic effects by reducing its elimination whereas they can decrease the toxic buildup of the metabolite of buprenorphine! Another example is benzodiazepines such as midazolam which are known to be metabolized by CYP3A4 enzyme. Its administration to patients taking semisynthetic and synthetic opioids can create a source of variability in toxicity and response. This situation is far more common than generally recognized. In fact, anesthetics and drugs routinely administered during the preoperative and perioperative periods can include lists containing midazolam and fentanyl. Potential drug interactions can then be expected in patients who are concurrently receiving inhibitors and substrates of CYP3A4 (e.g., ketoconazole, posaconazole), benzodiazepines (e.g., midazolam) and opioids [1, 46]. The contribution of CYP3A5 for metabolism of various drugs is also significant. In many cases, both CYP3A4 and CYP3A5 contribute to metabolism of the same drugs such as fentanyl. Therefore, it is possible that a patient has wild-type alleles for one enzyme and polymorphism in the other. This creates a challenge in interpretation of the genotyping results for the CYP3A4/5 families. Despite this concern, specific polymorphisms denoted as CYP3A4*1B and CYP3A5*3 have been found to be helpful in certification of postmortem fentanyl toxicity cases [25]. It is therefore recommended that for similar situations, both CYP3A4 and CYP3A5 be genotyped and their results be interpreted as an adjunct considering all other case evidence accordingly.

CYP2D6

Only 2–4% of the overall cytochrome composition in human hepatic tissue belongs to the CYP2D6 enzyme. Nevertheless, this enzyme, which is highly polymorphic, is responsible for metabolizing approximately 35% of all the drugs on the market [47]. The role of CYP2D6 in pharmacokinetics of many drugs of interest to forensic toxicologists has already been established [26, 30, 48, 49]. According to the Human Cytochrome P450 Allele Nomenclature Committee, there are over 120 reported base substitutions or polymorphisms reported by June 2009 [50]. Genotyping for these is routinely performed by commercially available kits capable of testing for 20 or less of these polymorphisms. Routinely, multiplexing or array-type techniques are best suited for CYP2D6 genotyping [51, 52]. Overall, the allele variants are designated by a * and a number. For example a *1 allele variant generally refers to the wild-type genotype. An allele variant of *2 is also expected to have normal activity whereas *3 through *8 and *11 through *15 genotypes denote no enzymatic activity. Partial activity is expected from those with allele designations of *9, *10, *11, and *41. Traditionally, four major genetically derived phenotypic designations have been described for this CYP2D6. Extensive metabolizers (EM) represent the norm for metabolic capacity. Genotypes consistent with the EM phenotype include two active CYP2D6 alleles (for example, *1/*1 or *1/*2) or one active and one partially active CYP2D6 allele. In general, extensive metabolizers can be administered drugs which are substrates of the CYP2D6 enzyme following standard dosing practices.

Increased caution may be appropriate for individuals having one partially active allele. Intermediate metabolizers (IM) may require lower than average drug dosages for optimal therapeutic response. Genotypes consistent with the IM phenotype are those with one active and one inactive CYP2D6 allele, one inactive and one partially active CYP2D6 allele, or two partially active CYP2D6 alleles. Poor metabolizers (PM) are at increased risk of drug-induced side effects due to diminished drug elimination or lack of therapeutic effect resulting from failure to generate the active form of the drug. Genotypes consistent with the PM phenotype are those with no active CYP2D6 genes. Ultrarapid metabolizers (UM) exhibit higher than average rates of metabolism. Genotypes consistent with the UM phenotype include three or more active CYP2D6 alleles due to duplication of an active allele. UMs are at increased risk of therapeutic failure as a result of increased drug elimination. Thus they may require an increased dosage of medications that are inactivated by CYP2D6. Alternatively, UMs may also be at increased risk of drug-induced side effects because of increased exposure to active drug metabolites. In this case, they may require lower than average doses.

In addition to the above-mentioned enzymes, there are several other genes such as the CYP2C19 and UGT subfamily which may be worth looking into during a case investigation. The National Academy for Biochemistry (NACB) has developed recommendations for the use of pharmacogenetics in forensic applications which are now closed for further comments and about to be published [53]. In addition, during the past couple of years, the College of American Pathologists has had proficiency testing surveys available for pharmacogenetic testing [54]. The remainder of this chapter will focus on the CYP3A4/5 and CYP2D6 genes by presentation of several cases illustrating the use of their genotypic information in working up toxicology cases.

Forensic Applications of Pharmacogenetics

In the discipline of forensic toxicology, results of drug screening activities as well as postmortem investigations are influenced by genetic differences in drug metabolism and elimination. We will focus on these areas in more detail below.

Interpretation of Urine Drug Screening Results

Toxicology screens have become very popular in both clinical and forensic toxicology disciplines. For clinical purposes, drug screens play an important role in the evaluation and treatment of the potentially poisoned patient. Other clinical applications include pain management, drug addiction treatment, and compliance testing. The forensic applications of drug screening are commonly used in workplace testing utilized by both private and governmental organizations. The consequences of

these results affect hiring practices, quality assurance, termination policies, and medical compensation for work-related injuries. Drug screening for other purposes such as driving under the influence and testing in athletes, students, and prisoners is also very popular. Obviously, the legal and social repercussions of a given test result are potentially devastating to the subject. In addition, the illicit drug use suggested by toxicological screens leads to employers routinely denying medical compensation to workers injured on the job should their hospital evaluation include a positive screening result. In many forensic situations, medical review officers (MRO) certify the drug screening results without any knowledge or evidence for an individual's ability to metabolize the drug in question. Added to this challenge is the fact that many drug screens are performed using immunoassays utilizing antibodies with differential cross-reactivities to the parent drug versus its metabolites. Otton et al. have demonstrated that the clearance of hydrocodone in the form of hydromorphone was 28.1 ± 10.3 mL/h/kg for patients with EM and 3.4 ± 2.4 mL/h/kg for those with PM genotypes for the CYP2D6 enzyme [55]. Therefore, in addition to the therapeutic efficacy of hydrocodone, the proportion excreted as its O-demethylated metabolite may have consequences on the urine opioid screening results [55, 56]. Another example is the metabolism of diazepam which is dependent on CYP2C19 activity [57]. Individuals with the PM genotype have prolonged half-lives for diazepam which are twice as long as those with the wild-type phenotype (88.3 ± 17.2 versus 40.8 ± 14.0 h, respectively). Obviously, benzodiazepine immunoassays with preferential cross-reactivities for the metabolites may have a reduced chance of detecting exposure to the drug. Combining analytical and pharmacogenetic screening was used in a case of an individual on oxycodone with continued negative drug screening results in the urine. Apparently, this individual had been on rifampin, which is a known inducer of CYP450 activity causing a very rapid half-life for the drug [58]. With the stated examples, it is apparent that alterations in metabolic capacity of drugs either due to polymorphisms or drug interactions can have consequences on the urine drug screening test results.

Pharmacogenetics in Forensic Investigations

Through presentation of several cases involving various different opioids, we will demonstrate the use of pharmacogenetic testing in establishing (or excluding) genetic differences in drug metabolism as a potential contributing factor to the cause of death. The field of forensic toxicology is in a great position to contribute to pharmacogenetics and its use in personalized medicine. When drugs are taken in "therapeutic" doses, toxicity and ultimately death are not generally expected. In cases where a patient dies after taking conventional doses of a drug or a combination of drugs, death investigation needs to be highly "individualized." This is best achieved by assessing the person's ability to metabolize the drugs through genotyping the DNA responsible for transcribing the relevant proteins and enzymes. Often, in individuals with reduced metabolic ability such as the IM or PM genotypes, the toxicity

is attributed to the parent drug. Alternatively, in those with the UM genotype, a higher than expected production of active metabolites can be the mechanism of toxicity. We will present several published and unpublished cases in which pharmacogenetics information was useful in determination of cause of toxicity or death.

Case Reports

We will initially focus on codeine and present several cases in which patients with various genotypes were investigated. We will then present an example for each of the other opioids, namely, oxycodone, fentanyl, and methadone.

Codeine is considered to be a weak opioid agonist, and is generally used for its analgesic and antitussive properties. The O-demethylation of codeine to morphine is by the CYP2D6 enzyme, and is considered to be important for its analgesic efficacy. Despite this, in PM subjects, respiratory depression and other side effects of opioid toxicity have been observed which are thought to be due to codeine itself. Therefore, it cannot be assumed that lack of CYP2D6 metabolic activity (by which codeine is converted to morphine) also results in the absence of side effects. The following cases demonstrate codeine toxicity in patients with different genotypes. In each of these, genotyping contributed to either the determination of the cause of death or was helpful in confirmation of the cause of death. Codeine is also metabolized by the CYP3A4 enzyme by *N*-demethylation to norcodeine which is equipotent to codeine.

Case 1: Codeine Intoxication in a Breast-fed Infant

This is the case of a newborn male infant who had developed lethargy at 7 days of age [59]. On day 11 after birth, the infant had been noted to have altered skin color and had reduced milk intake. The baby was finally transported to a hospital on day 13 for being cyanotic with no vital signs. Resuscitation efforts that had been initiated at home were unsuccessful and the patient was pronounced dead at the hospital. After ruling out various inborn errors of metabolism for conditions such as organic acidemias, fatty acid oxidative disorders, and thyroid issues, toxicological examinations were also performed. The postmortem blood sample had 70 ng/mL of morphine and 5.9 μg/mL of acetaminophen. The source of this blood sample was not mentioned in the report. This morphine concentration is approximately 6–7 times the therapeutic concentration seen in neonates receiving morphine for analgesia. The breast milk which he was being fed contained a morphine concentration of 87 ng/mL. This milk sample had been collected during the time his mother was taking half of the prescribed codeine dose during which she was somnolent and constipated. Pharmacogenetic analysis involved genotyping for CYP2D6 and UGT2B7 (catalyzing the morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) formation). The mother was considered to be an ultrarapid metabolizer since she

had CYP2D6 gene duplication (heterozygous with CYP 2D6*2A allele and a CYP 2D6*2×2 alleles). The father of the infant and the infant himself were EM with CYP2D6 *1/*2 genotypes. In addition, both the infant and his mother were homozygous for the UGT 2B7*2 gene known to be associated with increased M6G to morphine ratio. M6G is known be an active metabolite of morphine. Considering the genotype for the mother, it is apparent that she was converting more of the codeine to morphine due to her enhanced CYP2D6 activity. Additional morphine in her blood had led to her own somnolence and constipation. As a result, her milk also contained increased morphine which was fed to the infant. The clinical presentation of the infant prior to his death is consistent with opioid intoxication, also confirmed by the fact that the postmortem morphine values were in the toxic range.

Case 2: Codeine Intoxication in Twin Boys (Set A)

Codeine is widely used in the pediatric population for its antitussive as well as its analgesic properties. Compared to other opioids, it is generally regarded to have fewer side effects; therefore, it is frequently prescribed to younger children and neonates. This case involves codeine-induced toxicity in a recently published case of 3-year-old monozygotic twin brothers [48, 60]. They had been prescribed 10 mg of codeine to treat their cough following the diagnosis of upper respiratory infection. They were both administered codeine for 6 days. On the 6th day, 5 h after administration of the last dose, one of the twins was found to be apneic and had vomited. Their mother began resuscitation and the child was transferred to the pediatric intensive care unit. He was tachycardic, hypotensive, and had a Glasgow Coma Scale of 3. He had elevated leucocytes and was diagnosed with a tracheal viral infection. His aspiration pneumonia was treated by administration of antibiotics, and catecholamines were used to raise his blood pressure. After a few days, he eventually recovered with no further complications. Gas chromatography-mass spectrometry analysis of a serum sample collected 7.5 h after the last codeine dose resulted in total and free codeine concentrations of 489 and 179 ng/mL, respectively. The total and free morphine in the same sample were 312 and 33 ng/mL, respectively. The therapeutic serum concentration for codeine was listed as 56–129 ng/mL in small children. The concentration of morphine after codeine therapy has been mentioned to be 4.5 ± 2.1 ng/mL [60]. This particular case is consistent with codeine (and morphine) overdose leading to apnea, vomiting, and hypotension. Unfortunately, the second twin brother had been found dead in his bed at home shortly after the first twin was initially discovered to be in distress. Autopsy on the second twin revealed aspiration of gastric contents. Analysis of codeine and morphine were performed on several postmortem tissues and fluids on the second twin [60]. A serum sample obtained from the femoral vein resulted in a free codeine concentration of 547 ng/mL and a free morphine value of 150 ng/mL, respectively. The total and free codeine and morphine levels were also high in the cardiac blood. It is probable that respiratory depression and aspiration secondary to codeine (and the resulting morphine) overdoses led to the death of this twin brother. Genotyping

for CYP2D6 was used to investigate the reason for the elevation of both codeine and morphine. As expected, both twins had the same CYP2D6 genotypes which were considered to be wild types with no gene duplication. Therefore, they were categorized as extensive metabolizers thus ruling out the possibility of reduced metabolism due to genetic variation (i.e., being poor or intermediate metabolizer phenotypes). Accumulation of morphine was not attributed to CYP2D6 gene duplication since the children were not ultrarapid metabolizers. The pharmacogenetic data raises the suspicion that too much codeine had been administered to these children. Indeed, case investigation further revealed that the prescribed dose was 0.5 mL of the codeine solution resulting in 10 mg of the drug per dose. Sadly, their mother had administered the codeine to them by "drops." Each time, she administered 10 "drops" which were experimentally shown to range from 494 to 940 mg of codeine per dose. Authors had concluded that variations in "drop" size and imprecision in its measurements could have created the unfortunate overdose situation for these twins.

Case 3: Codeine Intoxication in Twin Boys (Set B)

The case of a second set of 3-year-old twin boys who had both died of respiratory depression following administration of codeine is presented. These children had undergone adenotonsillectomy operations within an hour of one another for severe obstructive sleep apnea syndrome (OSAS). Their operations had gone well with no complication. Both children had awakened, were extubated, and were stable. To control their surgical pain, each had received 5 mL of a codeine elixir containing 12 mg of codeine sulfate prior to discharge. Later on the same day, each child had further received two additional doses of the same codeine elixir at home. The recommended dose in children 3–6 years of age is 5 mL to be administered 3–4 times per day to be given every 3–4 h as needed (PRN). Interestingly, these children were being awakened to take their medication every 4 h. Several hours later, the first twin was noticed to be in respiratory distress and "choking," which eventually lead to acute cardiopulmonary arrest. CPR was initiated and the child was taken to the hospital. Resuscitation efforts were not successful and he was pronounced dead. While at the hospital with the first twin, the parents became concerned about the second twin who had been left in the care of a neighbor. The second twin was later on found to be unresponsive, had no pulse, and was cyanotic. He was resuscitated and eventually had his pulse reinstated. Ultimately, after 2–3 days of intensive care, the second twin also passed away. Autopsy performed on the first twin the morning after his death indicated that he had cerebral edema and airway froth. Toxicological analyses were performed on postmortem femoral blood, urine, vitreous fluid, and brain collected at autopsy from the first twin. Analysis of the peripheral blood sample resulted in total and free codeine concentrations of 740 and 540/mL, respectively. The total and free morphine levels in the same sample were 190 and 60 ng/mL, respectively. The concentrations of total and free codeine in the brain tissue were 530 and 500 ng/mL, respectively. Vitreous fluid contained primarily free codeine (300 ng/mL) and its

morphine concentration was <10 ng/mL. CYP2D6 genotyping on these twins showed that each had one functional allele (*2) and one nonfunctional allele (*4). Therefore, it is concluded that they were both intermediate metabolizers. It is clear that the codeine concentrations in twin A, and by extrapolation in twin B, were elevated at the time of cardiopulmonary arrest. Additional investigation into this case revealed that the mother had administered the correct amount of the drug to each child at each dosing time. The volume of unused elixir corroborated this conclusion. The twins had inherited the *4 from their mother and *2 from their father, since the mother was a carrier of *4 allele and the father was wild type carrying the *2 allele. Therefore, carrying a nonfunctional CYP2D6 allele more probably than not contributed in part to the reduced metabolism of codeine reflected by the toxic concentrations measured in the first twin at autopsy. Contribution of the extent of postoperative respiratory compromise or other variables to the demise of these children is not known.

Cases 4: Codeine in an Ultrarapid Metabolizer Child

In CYP2D6 rapid metabolizers, opioid toxicity after ingestion of codeine, hydrocodone, and oxycodone is possible due to the generation of too much morphine, hydromorphone, and oxymorphone, respectively. These metabolites are more potent than their parent counterparts. In a reported case, codeine elixir was used for managing pain in a 2.5-year-old boy who had undergone tonsillectomy operation [61]. He apparently had received four doses of codeine on postoperative day 1 and one more dose the next evening. Four hours later, the mother found the child unresponsive and apneic. The emergency team administered naloxone which led to some improvement; however, the child became apneic at the hospital and was intubated. After a couple of weeks, he was extubated and discharged in stable condition. CYP2D6 genotyping revealed that he had a copy of *1 allele and multiple copies of the *2 allele. Both *1 and *2 have enzymatic activity and having more than two copies renders the individual an ultrarapid metabolizer. In this case, use of pharmacogenetic information was found to be useful in implicating morphine as the cause of respiratory depression. However, the authors had not verified this finding by measuring the concentrations of codeine and morphine in this child.

Cases 5: Codeine in an Ultrarapid Metabolizer Adult

Another case of toxicity in individuals with multiple copies of CYP2D6 involves a 62-year-old gentleman with a history of leukemia who had presented with cough, fever, and dyspnea [62]. Since this patient was considered to be immunocompromised, bronchoalveolar lavage had been performed which revealed the presence of yeast. He was treated with two antibiotics (ceftriaxone and clarithromycin), an antifungal agent (voriconazole) and oral codeine (25 mg three times a day) for cough. His condition deteriorated on hospital day 4 and he became unresponsive. The last dose

had been administered 12 h prior to the changes in his level of consciousness. The patient's pupils were miotic and he had a Glasgow Coma Scale of 6 (no eye opening, no verbal response, and limb withdrawal after pain stimulation) on his initial neurologic examination. Administration of naloxone (0.4 mg repeated two times) resulted in a dramatic improvement in his level of consciousness. His plasma codeine concentration was 114 μg/L. The reference range for CYP2D6 extensive metabolizers has been reported to be 13–75 μg/L [62]. He was genotyped for CYP2D6 and CYP3A4, both of which are implicated in the metabolism of codeine. In addition, relative activities of CYP2D6 and CYP3A4 were assessed by administration of dextromethorphan and subsequent measurement of deconjugated dextrorphan excreted in the urine. The results of genotyping indicated that he had >3 copies of CYP2D6 which was confirmed by the phenotyping results assessed by the ratio of dextromethorphan and deconjugated dextrorphan. This patient was on a macrolide and an azole derivative to treat his infections. Both of these agents are known inhibitors of CYP3A4. It is believed that more of the codeine metabolized through the CYP2D6 route since CYP3A4 was inhibited, in this situation since there were multiple copies of CYP2D6 present to convert codeine to morphine. It is clear from this case that genotyping was useful in directing the investigation by focusing on codeine as a cause of decreased neurological function and opioid toxicity.

Case 6: Oxycodone in a Poor Metabolizer

The decedent was a 49-year-old white male, prescription drug abuser with a history of depression and posttraumatic stress disorder [49]. For treating his chronic back pain following surgery, OxyContin and Percocet were prescribed. He was an alcoholic. He attempted suicide once. Of the 60 oxycodone pills prescribed, only 12 had remained. His roommate, who saw him in the morning, found the decedent unresponsive after returning from work. Toxicological analysis showed subclavian blood, obtained within 24 h after death, with a concentration of oxycodone 0.437 mg/L, and without detection of alcohol and other drugs. Autopsy showed hepatic cirrhosis which might have impaired his drug metabolism. Molecular autopsy showed he was CYP 2D6*4 homozygous, corresponding to the poor metabolizer phenotype. This deficiency might have contributed to impaired metabolism of oxycodone, along with hepatic cirrhosis. Death certification was: cause of death, oxycodone overdose; and manner of death, accident.

Cases 7: Methadone in a Poor Metabolizer

The decedent was a 51-year-old white male with a 25-year history of heroin addiction for which he was enrolled in a methadone maintenance program [63]. On Friday, he was accompanied by his friend to the methadone clinic where he ingested his prescribed dose, and was given an extra dose for the weekend. He also bought illicit drugs near the clinic. His girlfriend confirmed that he was alive

at 7 a.m. on Sunday, and she found the decedent on Monday at 8 a.m., with a bottle of methadone nearby. The decedent had hepatitis C and hepatic cirrhosis. Toxicology showed the iliac blood methadone concentration to be 1.6 mg/L. Based on the case history, acute ingestion of methadone was likely, followed by postmortem interval of <24 h. Thus, the high methadone concentration was not due to postmortem redistribution. Molecular autopsy showed CYP 2D6*3 and *4 compound heterozygosity, corresponding to a poor metabolizer of methadone. Other toxicological findings included benzoylecgonine, 0.871 mg/L; propoxyphene, 0.32 mg/L; and diazepam, 0.12 mg/L. Autopsy finding included end-stage alcoholic liver disease. Death certification was: cause of death, mixed drug toxicity attributed to methadone, cocaine abuse, propoxyphene, and diazepam; and manner of death, accident.

Cases 8: Fentanyl in a Poor Metabolizer

The decedent was a 44-year-old white female, with a history of drug abuse (cocaine, marijuana, and pain medications), suicidal ideation, and psychiatric disorders [25]. In a previous attempt to obtain medications, she had cut her arm. After a rummage sale, she complained to her boyfriend about her knee pain, and obtained some narcotic patches. Later that evening, she seemed "goofy." She was found dead 24 h later. One Duragesic patch was attached to her arm, and another adhered to a blanket. Toxicology showed subclavian blood concentrations of : fentanyl and norfentanyl, 19 and 7.6 μg/L with a total of 26.6 μg/L; cyclobenzapine, 0.16 mg/L; tramadol, 0.06 mg/L; diphenhydramine, 0.08 mg/L; citalopram, 0.22 mg/L; and olanzapine, positive. Pharmacogenetic testing (i.e., molecular autopsy) showed: CYP3A4*1B heterozygous and CYP3A5*3 heterozygous. In these individuals, a reduced rate of fentanyl metabolism is expected. Therefore, according to the toxicology results and the genetic testing information, the death certification was issued as: cause of death, mixed drug toxicity attributed to fentanyl, diphenhydramine, citalopram, cyclobenzaprine, and tramadol; the manner of death was indicated to be an accident.

Techniques and Methods

Genetic variations in the genes which encode the drug-metabolizing enzymes may lead to normal, deficient, or higher enzyme activities. Such genetic variations can include SNPs, gene deletion, or gene duplications. For several enzymes such as CYP2D6, CYP2C9, and CYP2C19, there are several FDA-approved methodologies and kits available [64, 65]. In many of the techniques used for genotyping drug-metabolizing enzymes, DNA is initially isolated from blood or tissues and is amplified using PCR-based techniques. The variation in the gene sequence is then queried, using a variety of different methods. Restriction fragment length polymorphism (RFLP) has been considered to be the traditional approach in identifying known

mutations. This approach is limited by the fact that targeted polymorphisms have to be able to lead to alterations capable of forming a restriction site. The restriction sites will determine changes in the DNA fragmentation (restriction digestion) pattern which is identified on the gel [66]. In allele-specific amplification using real-time PCR, the product is amplified by PCR and its formation is detected [67]. Another approach is the multiplex PCR in which the target DNA sequence is amplified, and based on allele-specific primer extension technique, the simultaneous detection of multiple CYP2D6 variants is achieved [52]. There are many other methods such as HPLC, mass spectrometry, and sequencing which can detect variations in the DNA sequence. The detailed discussion of these methods is beyond the scope of this chapter and the reader is referred to other sources for more information [51, 66, 68].

Frequently, the question of "what sample type should be used for pharmacogenetic testing" comes up in forensic applications. DNA has been isolated from many different types of samples, ranging from dried blood spots or whole blood to various organs such as the liver. For some genes, sample condition is less of an issue than others. For example, many of the PCR products of CYP2D6 are large, and therefore sample integrity and DNA quality in the original sample is important. In the clinical environment, blood drawn in EDTA plasma tubes is recommended since DNA can be readily isolated from the buffy coat. On the other hand, collection of samples during autopsy is limited to getting a whole blood specimen or tissue. Since whole blood has been used with success for DNA isolation, it is the preferred specimen. However, if needed, tissue can also be used for isolation of DNA for pharmacogenetic testing.

Since many medical examiner offices and state laboratories do not routinely perform pharmacogenetic testing, it is recommended that they use referral laboratories for this type of determination. It is also crucial to consider the needed knowledge and skill set in order to properly interpret the results for forensic settings. As previously indicated, the drug-metabolizing gene testing is done as an adjunct to the overall process of case investigation. Most ideally, the interpretations should be performed by individuals with toxicology knowledge who have been specifically trained in pharmacogenetics. Obviously, molecular diagnostics experts often lack the pharmacology and toxicology background needed for forensic toxicology issues. If too much emphasis is put on the pharmacogenetic data without considering the fundamentals of forensic toxicology, the case can be easily misinterpreted. Therefore, understanding the drug concentrations and utilizing the pharmacogenetic data are best done by considering both items simultaneously. It is recommended that toxicologists consult with the pharmacogenetics experts and use the information as a piece of a larger puzzle.

In summary, when assessing therapeutic and toxic effects of opioids such as codeine which are often implicated in forensic cases, two distinct issues need to be considered. In the CYP2D6 poor metabolizers, not only opioid toxicity can be caused by the drug (e.g., codeine) itself, adequate pain relief mediated by its metabolite (e.g., morphine) is less likely [24, 69]. In a recent study, CYP2D6 genotyping was shown to predict only 50% of ultrarapid metabolizers subjects who carried gene duplication [27]. These subjects were better identified by dextromethorphan-based

phenotyping, which was able to distinguish 68% of the subjects on codeine with high morphine formation. When genotyping and phenotyping were combined, 88% of the high morphine formation subjects after administration of codeine were identified. On the other hand, CYP2D6 genotyping by itself was adequate enough to be able to predict insufficient morphine formation subjects. The point to consider when interpreting postmortem codeine cases is that determining the metabolic category for the patient is useful in establishing the underlying cause. For example, in twin set A (Case 2 discussed above), being an extensive metabolizer correlated with the finding that imprecision in "drop size" potentially leads to too much drug being administered. On the other hand, in twin set B (Case 3), carrying an inactive CYP2D6 allele explains toxic concentrations of codeine measured postmortem even if the correct dosing has been proven. Therefore, it is advised that genotyping be used as an additional piece of the puzzle when forensic toxicity cases are being investigated.

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Part II
CNS Drugs

Chapter 2
Drug Interactions with Benzodiazepines: Epidemiologic Correlates with Other CNS Depressants and In Vitro Correlates with Inhibitors and Inducers of Cytochrome P450 3A4

David E. Moody

Abstract The benzodiazepines are a class of a relatively large number of drugs that share a common chemical structure and have anxiolytic to sedative action on the central nervous system (CNS). They are chemically diverse, but share a classic structure that consists of a benzene fused to a seven-membered diazepine ring. Benzodiazepines are noted to have both pharmacodynamic and pharmacokinetic drug interactions. The former can be most devastating, and usually arise from co-exposure to another CNS depressant (e.g., ethanol, opioids, barbiturates, anesthetics). These have been associated with enhanced impairment and mortality, usually from respiratory depression. Pharmacodynamic interactions occur with all benzodiazepines and are not related to their structure. Pharmacokinetic interactions, on the other hand are highly structure dependent, as most arise from either inhibition or induction of the cytochrome P450s involved in the metabolism of the benzodiazepine. Numerous examples of pharmacokinetic interactions that alter the pharmacokinetics of the benzodiazepine have been reported and these are herein described for an assortment of drug. These interactions may have sufficient changes to significantly reduce efficacy (induction of metabolism), but toxicity from inhibition of metabolism was rarely seen at the therapeutic doses used in clinical studies. These consequences, however, could be magnified in the overuser. Numerous drug interactions between benzodiazepines and other drugs do occur; those with other CNS depressants are of greatest concern.

Keywords Benzodiazepines • Drug interactions • Drug metabolism • Respiratory depression

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General Information About Benzodiazepines

Introduction

The purpose of this chapter is to examine the drug interactions that occur with benzodiazepines and discuss the relevance of these interactions to the field of medicine in general with an emphasis on forensic toxicology. Because of the diverse nature of the benzodiazepines, some time has been taken to introduce this class of drugs. This introductory material has drawn upon some basic reference material and reviews [1–8], and is not otherwise referenced, except for specific points that did not come from these references. The primary literature will be more thoroughly cited in latter sections presenting evidence of interactions with other central nervous system (CNS) depressants and specific enzyme involvement in the metabolism of benzodiazepines and drug interactions.

The benzodiazepines are a class of a relatively large number of drugs that share a common chemical structure and have anxiolytic to sedative action on the CNS. Chlordiazepoxide was first introduced in the 1960s, followed by diazepam, flurazepam, and oxazepam. Since that time, a number of benzodiazepines have been introduced. In the 1999 edition of Martindale [7], at least 43 benzodiazepines were listed (Table 2.1). Most were found in the section on anxyolytic sedatives hypnotics and antipsychotics; one, clonazepam, was listed in the antiepileptics section. Of these 43 benzodiazepines only 15 have, or had, US manufacturers listed in the more recent online version of Martindales (Table 2.1) [9].

Most benzodiazepines are now made by more than one pharmaceutical house, or more than one subsidiary of a pharmaceutical house and therefore have more than one trade name. A single example of trade names has been listed in Table 2.1, along with an associated manufacturer.

To understand the importance of drug interactions with benzodiazepines, a basic understanding of their pharmacodynamic action is required, along with the related therapeutic use. In addition, because many of the drug interactions are of a pharmacokinetic nature, the chemical structure and metabolism of the benzodiazepines must be appreciated.

Pharmacodynamics (Briefly), Uses, and Adverse Effects of Benzodiazepines

Most of the effects of benzodiazepines arise from their action on the CNS. Within the CNS, the major molecular targets of the benzodiazepines are inhibitory neurotransmitter receptors directly activated by the amino acid, gamma-aminobutyric acid (GABA). Benzodiazepines have been shown to bind and modulate the major GABA receptor in the brain, $GABA_A$, while $GABA_B$ receptors are not altered by benzodiazepines. The $GABA_A$ receptor is an integral membrane chloride channel that mediates most of the rapid inhibitory

Table 2.1 Benzodiazepines listed in Martindales

Generic name	Representative trade name	Representative manufacturer	CAS #
Adinazolam	None	Upjohn, USA	37115-32-5
Alprazolam[a]	Xanax (others)	Upjohn, USA	28981-97-7
Bentazepam	Tiadipona	Knoll, Spain	29462-18-8
Bromazepam	Lexotan (others)	Roche, UK	1812-30-2
Brotizolam	Lendormin	B.I., Germany	57801-81-7
Camazepam[b]	Albego	Daker Farmasimos, Spain	36104-80-0
Chlordiazepoxide[a]	Librium (others)	Roche, USA	438-41-5
Cinolazepam	Gerodorm	Great, Australia	75696-02-5
Clobazam	Frisium	Hoechst, UK	22316-47-8
Clonazepam[a]	Klonopin (others)	Roche, USA	1622-61-3
Clorazepate[a]	Tranxene (others)	Abbott, USA	20432-69-3
Clotiazepam	Clozan (others)	Roerig, Belgium	33671-46-4
Cloxazolam	Akton (others)	Excel, Belgium	24166-13-0
Delorazepam	En	Ravizza, Italy	2894-67-9
Diazepam[a]	Valium (others)	Roche, USA	439-14-5
Estazolam[a]	Prosom (others)	Abbott, USA	29975-16-4
Ethyl Loflazepate	Victan (others)		29177-84-2
Etizolam	Depas (others)	Fournier, Italy	40054-69-1
Fludiazepam	Erispan	Sumitomo, Japan	3900-31-0
Flunitrazepam	Rohypnol (others)	Roche, UK	1622-62-4
Flurazepam[a]	Dalmane (others)	Roche, USA	1172-18-5
Halazepam[a,b]	Paxipam (others)	Schering, USA	23092-17-3
Haloxazolam	Somelin	Sankyo, Japan	59128-97-1
Ketazolam	Solatran (others)	SmithKline Beecham, Sweden	27223-35-4
Loprazolam	Dormonoct (others)	Hoechst Marian Russell, Belgium	61197-73-7
Lorazepam[a]	Ativan (others)	Biovail, USA	846-49-1
Lormetazepam	Loramet (others)	Wyeth, Greece	848-75-9
Medazepam	Rudotel	AWD, Germany	2898-12-6
Metaclazepam[b]	Talis	Organon, Germany	65517-27-3
Mexazolam	Sedexil	Medibial, Portugal	31868-18-5
Midazolam[a]	Versed	Roche, USA	59467-96-8
Nimetazepam[b]	Ermin	Suitomo, Japan	2011-67-8
Nitrazepam	Mogadon (others)	ICN, UK	146-22-5
Nordazepam	Nordaz (others)	Boucharo-Recordati, France	1088-11-5
Oxazepam[a,c]	Serafax (others)	Wyeth, India	604-75-1
Oxazolam	Serenal	Sankyo, Japan	24143-17-7
Pinazepam	Domar (others)	Teoforma, Italy	52463-83-9
Prazepam[a,c]	Centrax (others)	Parke-Davis, Germany	2955-38-6
Quazepam[a]	Doral (others)	Questcor, USA	36735-22-5
Temazepam[a]	Restoril (others)	Novartis, USA	846-50-4
Tetrazepam	Myolastan (others)	Sanofi Aventis, France	10379-14-3
Tofisopam	Grandaxin	Hung	22345-47-7
Triazolam[a]	Halcion	Pharmacia Upjohn, USA	28911-01-5

Note: Benzodiazepines listed in the 32nd edition of "Martindale: The Complete Drug Reference (1999)" [7]. When more than one trade name was listed (noted as "other"), either the USA or most common one was chosen; a representative manufacturer was selected for listing. Listed in latest online edition [9] as: [a]having a US manufacturer; [b]manufacturing suspended; [c]manufacturing suspended in USA, but still made in other countries

Table 2.2 Uses of benzodiazepines

1. Anxiety (27)[a]
2. Insomnia (26)
3. Presurgery/sedation (8)
4. Epilepsy/seizures (7)
5. Alcohol withdrawal (4)
6. Muscle spasms (3)
7. Panic disorder (2)
8. Depression (2)

[a] The number in parentheses represents the number of benzodiazepines listed in Martindale that are used to treat this disorder

neurotransmission in the CNS. Benzodiazepines, unlike barbiturates that also bind $GABA_A$, act only in the presence of GABA. Typical benzodiazepine agonists increase the amount of chloride current generated by $GABA_A$ activation, potentiating the effect of GABA throughout the CNS. Bicuculline, an antagonist of $GABA_A$, reduces the behavioral and electrophysiological effects of benzodiazepines, and a benzodiazepine analog, flumazenil, that potently and selectively blocks the benzodiazepine binding site, is used clinically to reverse the effects of high doses of benzodiazepines [4].

These CNS depressive effects result in anxiolytic, muscle relaxant, hypnotic, anti-grade amnesia, anticonvulsant, and sedative effects that define the therapeutic uses of benzodiazepines (Table 2.2). While the proper dose of any one benzodiazepine will produce many of these effects, some benzodiazepines are more appropriate for certain uses than others. In large part, this is dictated by the therapeutic half-life of the drug. Benzodiazepines are generally classified as short- (0–6 h), intermediate- (6–24 h), or long-acting (>24 h); some texts, however, will just use short- (0–24 h) and long-acting (>24 h) designations. Benzodiazepines used as anticonvulsants are long acting and have rapid entry into the brain. Short- to intermediate-acting benzodiazepines are favored for the treatment of insomnia. Short-acting benzodiazepines are used as preanesthetic agents for sedation prior to surgery. Long-acting or multidose shorter-acting benzodiazepines are generally used as anxiolytics. The use of benzodiazepines listed in Martindale, along with their half-life, route(s) of administration, and normal range of doses is presented in Table 2.3.

Drowsiness, sedation, and ataxia are the most frequent adverse effects of benzodiazepine use. They generally decrease on continued administration and arise from the CNS depressive effects of benzodiazepines. Less common adverse effects include vertigo, headache, mental depression, confusion, slurred speech, tremor, changes in libido, visual disturbances, urinary retention, gastrointestinal disturbances, changes in salivation, and amnesia. Rare events include paradoxical excitation leading to hostility and aggression, hypersensitivity reactions, jaundice, and blood disorders. With very high doses, hypotension, respiratory depression, coma, and occasionally death may occur.

Table 2.3 Uses of benzodiazepines listed in Martindale

Generic name	Half-life (h)[a]	Route(s) of administration	Usual dose (mg)	Uses[b]
Adinazolam	Short	–	–	1, 8
Alprazolam	11–15	Oral	0.75–1.5	1, 8
Bentazepam	–	Oral	25	1, 2
Bromazepam	12–32	Oral	3–18	1, 2
Brotizolam	4–8	Oral	0.25	2
Camazepam	–	Oral	10	2
Chlordiazepoxide	5–30, 48–120[c]	Oral, iv, im	25–100	1, 2, 3, 5, 6
Cinolazepam	–	–	–	2
Clobazam	18, 42[c]	Oral	20–30	2, 4
Clonazepam	20–40	Oral, iv	0.25–1	4, 7
Clorazepate	48–120[c]	Oral, iv, im	15–90	1, 4, 5
Clotiazapam	4–18	Oral	5–60	1, 2
Cloxazolam	Long	Oral, im	8–12	1, 3
Delorazepam	Long	Oral, im	0.5–6	1, 2, 3, 4
Diazepam	24–48, 48–120[c]	Oral, iv, im	5–30	1, 2, 3, 4, 5, 6
Estazolam	10–24	Oral	1–2	2
Ethyl Lorazepate	Long	Oral	1–3	1
Etizolam	Short	Oral	3	1, 2
Fludiazepam	Short	Oral	–	1
Flunitrazepam	16–35	Oral, iv	0.5–2	2, 3
Flurazepam	47–100	Oral	15–30	2
Halazepam	Short	Oral	20	1
Haloxazolam	Short	Oral	5	2
Ketazolam	Long	Oral	15–60	1
Loprazolam	4–15	Oral	1–2	2
Lorazepam	10–20	Oral, iv, s.l.	1–6	1, 2, 3, 4
Lormetazepam	11	Oral	0.5–1.5	2
Medazepam	Long	Oral	10–20	1
Metaclazepam	Short	Oral	15	1
Mexazolam	–	Oral	0.5	1
Midazolam	2–7	iv, im	2.5–7.5	3
Nimetazepam	Short	Oral	3	2
Nitrazepam	24–30	Oral	5–10	2, 4
Nordazepam	48–120	Oral	15	1, 2
Oxazepam	4–15	Oral	15–30	1, 2, 5
Oxazolam	Long	Oral	10	1
Pinazepam	Long	Oral	5–20	1, 2
Prazepam	48–120[c]	Oral	30–60	1
Quazepam	39, 39–73[c]	Oral	15	2
Temazepam	8–15	Oral	10–40	1, 3
Tetrazepam	–	Oral	25–50	6
Tofisopam	–	Oral	150	1
Triazolam	1.5–5.5	Oral	0.125–5	2

[a]If half-lives were not given, they were often referred to as short- or long-acting
[b]See Table 2.2 for the number corresponding to different uses
[c]Half-life for active metabolite

Daily benzodiazepine use has been associated with dependence, tolerance, and after discontinuation, withdrawal symptoms in many individuals. Tolerance to the effects of benzodiazepines is a highly debated topic. It appears to occur in some individuals and may not occur in others. The likelihood of dependence appears higher in individuals with a history of drug or alcohol dependence and personality disorders. High doses and intravenous injection are used for their euphoric effects. Because development of dependence cannot be easily predicted, abrupt discontinuation of use is not recommended. Rather the dose should be tapered. Symptoms of withdrawal include anxiety, depression, impaired concentration, insomnia, headache, dizziness, tinnitus, loss of appetite, tremor, perspiration, irritability, perceptual disturbances, nausea, vomiting, abdominal cramps, palpitations, mild systolic hypertension, tachycardia, and orthostatic hypotension. If long-term use of benzodiazepines occurs, professional assisted withdrawal is recommended.

Basic Pharmacokinetics

The benzodiazepines are generally lipophilic drugs. Within the class, however, lipophilicity measured as the oil:water coefficient can differ over a 50-fold range. Due to their lipophilicity the benzodiazepines have relatively high plasma protein binding (70–99%) and relatively large volumes of distribution (0.3–22 L/kg) (Table 2.4). In general, the percent plasma protein binding and the volume of distribution increase as does the oil:water partition coefficient.

The differences in lipophilicity can have a major impact on the pharmacokinetics of the benzodiazepine. Diazepam is regarded as a long-acting benzodiazepine. When diazepam is given as a single dose, however, it rapidly redistributes to non-plasma (lipid) compartments, which is referred to as the α elimination phase. It then slowly distributes back into the plasma compartment at subtherapeutic concentrations with a long terminal elimination half-life. Therefore, single doses of diazepam can be used as a preanesthesia medication, while daily dosing will result in accumulation during the terminal elimination phase and provide long-acting therapy.

The benzodiazepines are well absorbed from the gastrointestinal tract, which allows for oral dosing of benzodiazepines (Table 2.3). As described in more detail in the Section on metabolism, most will also undergo extensive first-pass metabolism, some to such an extent that parent drug is only detected at very low concentrations in blood (or blood-derived) samples. The plasma concentration of benzodiazepines, or their primary pharmacodynamically active metabolites, correlates well with the dose of benzodiazepine administered (Fig. 2.1).

As a class, the benzodiazepines share many properties. There are structural differences between them, and these differences will effect the manner in which the benzodiazepine is metabolized, and thereby have an impact on their individual susceptibility to drug interactions.

Table 2.4 The percent of plasma protein binding and volume of distribution (V_d) of some benzodiazepines

Benzodiazepine	% Bound	V_d (L/kg)	Source
Alprazolam	71	0.7	a
Bromazepam	70	0.9	b
Chlordiazepoxide	96	0.3	a
Clobazam	85	1.0	b, c
Clonazepam	86	3.2	a
Clotiazepam	99	–	c
Diazepam	99	1.1	a
Estazolam	93	–	c
Flunitrazepam	78	3.3	a
Flurazepam	97	22.0	a
Halazepam	–	1.0	b
Lorazepam	91	1.3	a
Midazolam	95	1.1	a
Nitrazepam	87	1.9	a
Nordazepam	98	0.8	a
Oxazepam	98	0.6	a
Prazepam	–	13.0	b
Quazepam	95	–	c
Temazepam	98	1.1	a
Triazolam	90	1.1	a

The source of information was a [5]; b [6]; and c [7]

Chemistry and Metabolism of Benzodiazepines

Chemistry of Benzodiazepines

The classic structure of benzodiazepines (Fig. 2.2) consists of a benzene (A ring) fused to a seven-membered diazepine (B ring). In all but two of the commercially available benzodiazepines, the nitrogens in the diazepine ring are in the 1,4-position. Clobazam has nitrogens in the 1,5-position of the diazepine ring; tofisopam has nitrogens in the 2,3-position of the diazepine ring (Fig. 2.3). In addition, most commercially available benzodiazepines have an aryl substituent (C ring) at the 5-position of the diazepine ring. Therefore, with the exception of clobazam and tofisopam, these are 5-aryl-1,4-benzodiazepines.

Following the initial synthesis of chlodiazepoxide by Sternbach in 1957, and its introduction as a therapeutic agent in 1961, a number of benzodiazepines have been introduced onto the market. The initial modifications involved changes in the substituents on the diazepine ring. Modifications along this line first led to the development of diazepam, flurazepam, and oxazepam. These have continued through the years, leading to a number of 1,4-benzodiazepines (Table 2.5). Substitution of the benzene with a thieno group produced the 1,4-thienodiazepines

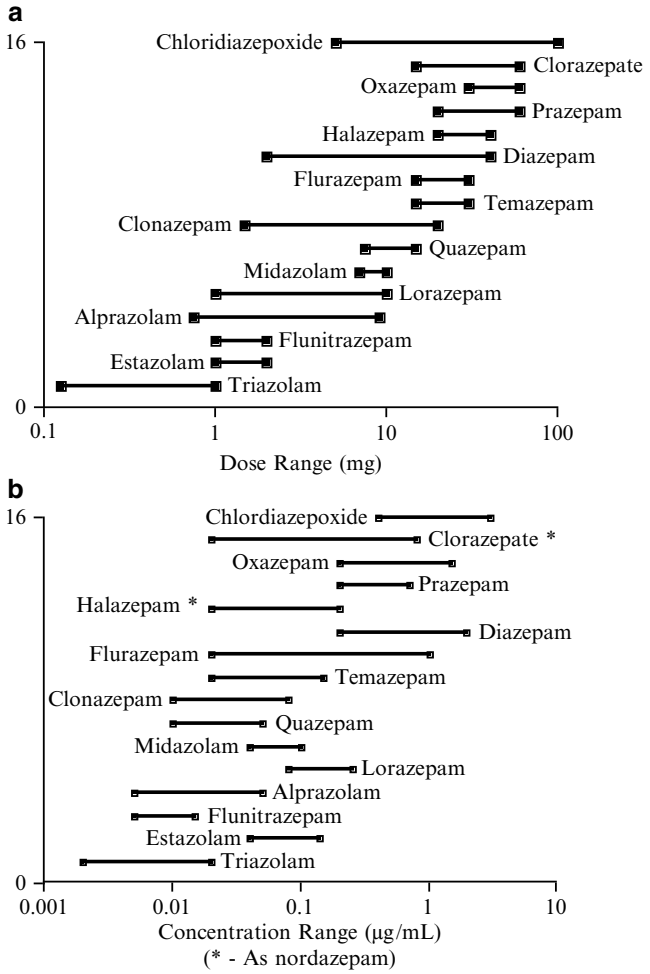

Fig. 2.1 The range of therapeutic doses (**a**) and plasma concentrations (**b**) of selected benzodiazepines. *In (**b**), these concentrations are for the primary metabolite, nordiazepam

(Figs. 2.2 and 2.3, Table 2.6). Annelation of an oxazolo (Fig. 2.2, Table 2.6) or oxazino group (ketazolam in Fig. 2.3, Table 2.6) at the 4,5-position of the diazepine has been used and the newer benzodiazepines have 1,2 annealed triazolo or imidazo groups (Fig. 2.2, Table 2.6). While most benzodiazepines have a phenyl substituent at the 5-position of the diazepine ring, bromazepam has a 2-pyridinyl substituent, and tetrazepam has a 1-cyclohexen-1-yl substituent at this position (Fig. 2.3, Table 2.6). Bentazepam, with a benzylthieno group fused to the diazepine ring, and brotizolam with both the thieno and triazolo groups are unique 1,4-thienodiazepines (Fig. 2.3, Table 2.6).