Understanding the Metabolism of Codeine is Important for the Clinician and the Forensic Pathologist

Introduction

Recently, an advisory committee to the FDA has recommended codeine be contraindicated for pain and cough management in children and adolescents due to concerns for respiratory depression and death. This FDA warning was also extended to prescriptions of codeine to nursing mothers.

In Europe, codeine is contraindicated in children under age 12 for cold or cough, and the European Medicines Agency recommends against using the drug in those age 12 to 18 years-of-age who have breathing difficulties.

We need to explore the foundation for the necessity of this recommendation.

General Information

Codeine is a narcotic analgesic and is considered a weak opioid; typically given with acetaminophen; is not considered effective for severe pain; and 33% of children are not codeine responders.

Codeine is one of the naturally occurring alkaloids found in opium, which was first isolated in 1832. Depending on where the poppies are grown, samples of opium have a codeine content that varies between 0.7 to 2.5%. Most of the codeine consumed in antitussive and analgesic mixtures is of semisynthetic origin, produced through 3-O-methylation of morphine. It is considered to be 1/10 to 1/6 as potent an analgesic as morphine on a weight basis, but is quite effective as an antitussive. Codeine is found in numerous proprietary preparations in combination with non-narcotic analgesics, antihistamines and other drugs. The DAWN (Drug Abuse Warning Network) report for 1990 listed 501 codeine related deaths. By 1999 the number of codeine related deaths reported by DAWN had increased to 1395.

Although substantial quantities of codeine are consumed annually, there are still questions concerning codeine metabolism and toxicity that are not understood. Although, it has been understood for half a century the pain relieving properties of codeine are due to its conversion to morphine (Sanfilippo, 1948), the mechanisms by which this occurs is still not completely known. What is of considerable importance, and may account for some of the unexpected cases of respiratory depression and death in children is there is considerable variation in the inter- and even intra-individual ability to metabolize codeine (Lee et al., 1997; Caraco et al., 1999). This variation can have unexpected consequences as shown by recent reports of severe adverse events in breastfed infants, whose mothers had altered metabolism of codeine. It was this altered metabolism, which led to the above FDA warning on prescription of codeine to nursing mothers.

Metabolism and Excretion

The major pathways for the metabolism of codeine occur in the liver, although some metabolism occurs in the intestine and brain. Approximately 50 to 80% of codeine is converted to codeine-6-glucuronide by UGT2B7.
UGT2B7 (UDP-Glucuronosyltransferase-2B7) is a phase II metabolism isoenzyme found to be active in the liver, kidneys, epithelial cells of the lower gastrointestinal tract and has also been reported in the brain. UDP-Glucuronosyltransferase-2B7 is encoded by the *UGT2B7* gene. UGTs play a major role in the conjugation and subsequent elimination of potentially toxic xenobiotics (chemical substances found within any biologic organism that are not normally produced or expected to be present in that organism, such as antibiotics, analgesics, opioids, etc.) as well as endogenous compounds, such as 3,4-catechol estrogens and estriol. UGT2B7 is the major enzyme isoform for the metabolism of morphine to the main metabolites, morphine-3-glucuronide (M3G), which has no analgesic effect, and morphine-6-glucuronide (M6G), which has an analgesic effect but not as strong as morphine. Because of its role as a metabolism isoenzyme, any alteration in UGT2B7 activity can significantly affect both the effectiveness and side-effects of morphine, as well as some related opiate drugs, such as codeine.

Codeine-6-glucuronide has a similar affinity as codeine for the mu opioid receptor, coded for by the OPRM1 gene. However, although like morphine, codeine is an agonist of the mu opioid receptor (OPRM1), it is less potent agonist of the OPRM1 receptor when compared to morphine, and thus was considered a safer alternative in an outpatient setting.

OPRM1 (Opioid Receptor, Mu 1) is a Protein Coding gene, which encodes for one of at least three opioid receptors in humans: mu1, mu2 and mu3. The mu opioid receptor (MOR) is also known as OP3 and MOP. The MOR is the principal target of endogenous opioid peptides and opioid analgesic agents such as beta-endorphin, enkephalins, morphine, codeine, etc. What is also of importance is MOR has an important role in dependence to other drugs of abuse, such as nicotine, cocaine, and alcohol via its modulation of the dopamine system. The NM_001008503.2:c.118>G allele has been associated with opioid and alcohol addiction and variations in pain sensitivity, although, evidence for it having a causal role is conflicting. Multiple transcript variants encoding different isoforms have been found for this gene.

The mu opioid receptor, is a member of the opioid family of G-protein-coupled receptors that also includes kappa, delta and NOP (nociceptin receptor, also known as the nociceptin/orphanin FQ (N/OFQ) receptor or kappa-type 3 opioid receptor) receptors. NOP is encoded by the *OPRL1* (opioid receptor-like 1) gene.

muOpioid receptors are distributed throughout the neuraxis (neocortex, thalamus, nucleus accumbens, hippocampus, amygdala) and in the peripheral nervous system (myenteric neurons and vas deferens). muOpioid receptors have been implicated in respiration, cardiovascular functions, feeding, learning and memory, intestinal transit, locomotor activity, thermoregulation, hormone secretion and immune functions. The gene encoding the mu opioid receptor has been localized to chromosome 6 (6q24-q25).

Approximately 10 to 15% of codeine is N-demethylated to norcodeine by CYP3A4. Norcodeine has a similar affinity with the mu opioid receptor as codeine.

CYP3A4 (Cytochrome P450 3A4) is found primarily in the liver and in the intestines. Recently it as also been identified in the brain, however its role in the central nervous system is still unknown. Its purpose is to oxidize small foreign organic molecules (xenobiotics), such as toxins or drugs. It is involved in the metabolism of approximately
half the drugs that are used today, including acetaminophen, codeine, morphine, cyclosporin, diazepam, and erythromycin. It also metabolizes some steroids and carcinogens. The cytochrome P450 proteins are monoxygenases that catalyze many reactions involved in drug metabolism, as well as the synthesis of cholesterol, steroids, and other lipid components. CYP3A4 is the most common and the most versatile of the cytochrome P450 family of oxidizing enzymes. As is true of all members of this family, it is a hemoprotein (a protein containing a heme group with an iron atom). The CYP3A4 protein is encoded by the CYP3A4 gene, which is part of a cluster of cytochrome P450 genes on chromosome 7q21.1.

What is of considerable importance to remember is the alleles of CYP3A4, specifically, CYP3A4*6 (an A177776 insertion) and CYP3A4*17 (F189S), have minimal function as compared to the wild-type CYP3A4 (the wild type allele is associated with normal enzyme activity). Both of these SNPs (single nucleotide polymorphisms) lead to a marked decrease in the metabolism of certain ligands. SNPs are the most common type of genetic variation among people. SNP refers to a difference in a single DNA building block, called a nucleotide. It is these alleles of CYP3A4 which may account for some of the unexpected cases of respiratory depression and death, especially in newborns and children.

A ligand in biochemistry and pharmacology, refers to a substance that forms a complex with a biomolecule to serve a biological purpose. In protein-ligand binding, the ligand is usually a signal-triggering molecule binding to a site on a target protein. The binding typically results in a change in conformation of the target protein. For example, ligand binding to a receptor alters its chemical conformation. The conformational state of a receptor protein determines its functional state. Ligands include substrates, inhibitors, activators, and neurotransmitters. The tendency or strength of binding is called affinity.

Between 0 to 15% of codeine is O-demethylated to morphine, which is the most active metabolite and has a 200 fold greater affinity for the mu opioid receptor compared to codeine. This metabolic reaction is accomplished by CYP2D6. Remember, the pain relieving properties of codeine, which vary between 1/10 to 1/6 as those of morphine, are due to its conversion to morphine (Sanfilippo, 1948), CYP2D6 (Cytochrome P450 2D6) is an enzyme encoded by the CYP2D6 gene. It is primarily expressed in the liver, however it is also highly expressed in areas of the CNS, including the substantia nigra.

CYP2D6, belongs to the cytochrome P450 mixed function oxidase system. It is responsible for the metabolism and elimination of approximately 25% of clinically used drugs, in a process called O-demethylation (demethylation is a chemical process by which a methyl group [CH₃] is removed from a molecule. O-demethylation refers to cleavage of ethers, codeine is morphine 3-methyl ether).

What is of particular importance to the clinician concerning their patients and the forensic pathologist regarding the determination of cause and manner of death is the considerable variability in the efficiency and amount of CYP2D6 enzyme produced between individuals. Consequently, for the drugs metabolized by CYP2D6, certain individuals will eliminate these drugs quickly (ultrarapid metabolizers, UMs) while others slowly (poor metabolizers, PMs). If a drug is metabolized too quickly, it may decrease
the drug’s efficacy while if the drug is metabolized too slowly, toxicity may result from
the parent drug, which may lead to respiratory depression, and in some cases death.
Hence, for the clinician, the dose of the drug may have to be adjusted to take into
account the speed at which it is metabolized by CYP2D6. Likewise, the forensic
pathologist must take such variance in metabolism when considering cause and manner
of death in a suspected death due to drug intoxication.
Another factor which must be kept in mind, some drugs may function as inhibitors of
CYP2D6 activity or inducers of CYP2D6 enzyme expression, which will lead to
decreased or increased CYP2D6 activity respectively. If such a drug is taken at the
same time a second drug that is a CYP2D6 substrate, the first drug may affect the
elimination rate of the second through what is known as drug-drug interaction.
The CYP2D6 gene is located near two cytochrome P450 pseudogenes on chromosome
22q13.1.
It is also important to remember, CYP2D6 shows the largest phenotypical variability
among the CYPs, which is primarily due to genetic polymorphism. The genotype
accounts for normal, reduced, and non existent CYP2D6 function in subjects.
Pharmacogenomic tests are available, such as the administration of debrisoquine (a
selective CYP2D6 substrate) with subsequent plasma concentration assay for the
debrisoquine metabolite (4-hydroxydebrisoquine), to identify patients with variations in
the CYP2D6 allele (see discussion under Genetic Polymorphism). Following such a
test, the CYP2D6 function in any patient can be described as follows:
a. Poor metabolizer (PM): little or no CYP2D6 function. Approximately 10% of people
   are PMs.
b. Intermediate metabolizer (IM): metabolize drugs at a rate somewhat between poor
   and extensive metabolizers. Approximately 10% of people are IMs.
c. Extensive metabolizer (EM): normal CYP2D6 function. Approximately 78% of
   people are EMs.
d. Ultrarapid metabolizer (UM): multiple copies of the CYP2D6 gene are expressed,
   and therefore greater than normal CYP2D6 function. Approximately 2% of people
   are UMs.
What determines an individuals phenotypic activity is their highest functioning CYO2D6
allele. EMs possess at least one fully functional CYP2D6 allele, and are thought to be
phenotypically normal. IMs (two reduced function or one reduced and one non-
functional allele) and PMs (two non-functional alleles) are not able to metabolize
CYP2D6 substrates as well as their EM counterparts, and may be at increased risk for
adverse effects resulting from higher plasma levels of the parent drug, or lack of efficacy
resulting from an inability to form an active metabolite. UMs possess multiple functional
copies of a single CYP2D6 gene. The CYP2D6 copy number has been found to be
from 2 to 13. Each functional copy of CYP2D6 that is present increases the rate of
metabolism of CYP2D6 substrates significantly. CYP2D6 reduced function alleles
include: *9, *10, *17, *29, and *41. CYP2D6 nonfunctioning alleles which will encode for
CDP2D6 enzymes which have little or no metabolic activity include: *3, *4, *5, *6, *7, *8,
Thus, the type of CYP2D6 function of a patient influences their response to different
doses of drugs the CYP2D6 metabolizes. The nature of the effect on the drug response
dePENDS not only on the type of CYP2D6 function, but also on the extent to which
processing of the drug by CYP2D6 results in a chemical that has an effect that is similar, stronger, or weaker than the original drug, or no effect at all. This is the issue when codeine undergoes O-demethylation to morphine. The extensive and ultrarapid metabolizers will have an exaggerated response to codeine, with the UMs having more of an exaggerated response than EMs, with stronger side effects, which may include respiratory depression and in some cases death. On average EMs (extensive hydroxylators) will convert approximately 6% of a given dose of codeine (demethylation followed by conjugation) to morphine. If however, the patient is a poor metabolizer, they will still produce morphine from codeine, but much less than the EMs or UMs (less than 1% conversion). Thus, the concentrations of morphine and its metabolites in their plasma will be so low as to be not measurable. Having said that, these metabolites are still formed, and they do appear in the urine. Screening test for opiates will be positive in both PMs, EMs and UMs, however, they will remain positive significantly longer in EMs (mean, 33 hours for EMs vs. 17 hours for PMs). These findings have significant forensic implications, for some individuals will have more psychoactive substances in their blood than simple measurements of morphine or codeine levels would indicate. The opposite is also true. Individuals who are PMs (slow hydroxylators) would have little or no morphine detectable in their blood, if the only drug taken was codeine. Morphine will be detectable in their urine, however, only for a short time. There is another important factor to remember, the presents of codeine in the patients urine should not be presumed to be evidence for anything but the ingestion of codeine. However, the presents of trace amounts of morphine, is accounted for by the metabolic conversion of codeine to morphine.

Another factor that affects CYP2D6 variability is ethnicity. The prevalence of CYP2D6 PMs is approximately 6 to 10% in white population but is lower in most other ethnic groups such as Asians in which it is 2%. PMs are found mainly in Europe. In African-Americans, the frequency of PMs is greater than for whites. The majority allele in this population is the functional \textit{CYP2D6*1} (70%), but the remaining alleles include the nonfunctional \textit{CYP2D6*4} and \textit{CYP2D6*5} variants that largely account for the PMs in these populations (Ingelman-Sundberg et al., Pharmacogenomics journal, 2005). The occurrence of CYP2D6 UMs appears to be greater among Middle Eastern, North African populations and Oceania (Oceania refers to a region centered on the islands of the tropical Pacific Ocean [subregions of Melanesia, Micronesia, and Polynesia although, some classify Oceania as the entire insular region between Southeast Asia and the Americas, including Australasia and the Malay Archipelago]). It is estimated UMs are present in up to 28% of North Africans, Ethiopians, and Arabs; up to 10% of Caucasians; 3% in African Americans, and up to 1% in Hispanics, Chinese, and Japanese (Roxane Laboratories, 2010).

In Asians and in individuals of Asian descent, only about 50% of \textit{CYP2D6} alleles are functional, with the reduced function \textit{CYP2D6*10} variant being very common (~40%). As a result, Asians are more likely to be IMs than Caucasians (Bradford et al., Pharmacogenomics, 2002). Individuals who have at least one copy of a functional allele (*1 or *2), or two partially functioning alleles, have a phenotypically normal response to codeine (EMs). About 77
to 92% of patients have this phenotype (Crews, et al., Clinical pharmacology and therapeutics, 2012)
Ethnic differences in the gene encoding UGT2B7 have also been identified, however, these have not been associated with clinical differences in enzyme activity (Bhasker et al., 2000).

Age and sex have also been shown to affect opioid metabolism. Reduced clearance of morphine, codeine, fentanyl, and oxymorphone has been reported in older patients. Oxycodone concentrations are approximately 25% higher in women than in men after controlling for differences in body weight, making it important for physicians to consider the patient’s sex when prescribing this opioid in adults (Purdue Pharma LP, 2007).

Approximately 60 to 70% of the morphine is converted to glucuronides, morphine-3-glucuronide (M3G), while 5 to 10% is converted to morphine-6-glucuronide (M6G) and then excreted by the kidneys. These reactions are primarily catalyzed by UGT2B7 in the liver. UGT1A1 may have a minimal role in the formation of M3G. Both UGT1A1 and UGT1A8 are capable of catalyzing the formation of M6G in vitro and so it is believed they contribute to this pathway. M3G has almost no affinity for the mu receptors in the brain and thus has no analgesic properties. However, M6G selectively binds to both mu1 and mu2 receptors, which is thought to explain its analgesic properties, as well as its ability to depress respirations. M6Gs selective binding to the mu receptors is also why the ratio of morphine to M6G is considered an important indicator of analgesic effect.

There is increased interest in transporters, such as P-glycoprotein or organic acid transporters, which move drugs across membranes including the gut and the blood brain barrier. It is believed transporters play an important role in the pharmacokinetics of codeine and morphine as they influence clearance of codeine, morphine and their metabolites. It is believed not only in the case of codeine and morphine, but in many other commonly used and important drugs, they are carried by these transporters and may be subject to both induction and inhibitory interactions. Although some genetic polymorphisms have been identified, such as in the transporters ABCB1, ABCC2m ABCC3 and SLCO1B1, there is no evidence of a clearly defined clinical association.

Genetic Polymorphisms

Polymorphisms of phase 1 cytochrome P450 (CYP450) metabolizing enzymes was first described some 40 years ago when it was noted a few of the subjects given the antihypertensive drug debrisoquine had extreme falls in blood pressure which were related to abnormally high plasma drug concentrations. It was determined that debrisoquine, as well as over 70 other drugs were metabolized by the enzyme, CYP2D6. This is one of a series of cytochrome P450 enzymes found in the liver endoplasmic reticulum. Each of these enzymes has a unique, but often overlapping, range of drug substrates. CYP2D6 is highly polymorphic with over 90 variants known. CYP2D6*1 is the wild type allele and is associated with normal enzyme activity. The *2 allele has near normal
enzyme activity (80% of the wild type) (Bradford LD., Pharmacogenomics, 2002). EMs carry an autosomal dominant wild type gene and may be homozygous or heterozygous for this allele. PMs have two abnormal alleles and over 80 genotypic variants accounting for approximately 98% of phenotypic PMs. These include point mutations, insertions and gene deletions, which result either in no enzyme or very limited quantities of stable enzyme being produced. Depending on the nature of the allelic variations the heterozygous EMs they may be classified as IMs. Today genetic testing is available for approximately 30 variants of the CYP2D6 alleles.

Toxicity

The acutely lethal dose of codeine for an adult has been estimated at 0.5 to 10 g. Doses of this magnitude may cause unconsciousness and convulsions, and death due to respiratory failure within 2 to 4 hours. One study has indicated postmortem blood codeine concentrations in 8 adults succumbing to codeine overdosage ranged from 1.4 to 5.6 mg/L (Wright et al., 1975). A newborn infant, whose mother was taking 60 mg of codeine per day, died after 2 weeks of breastfeeding with a postmortem blood morphine level of 0.09 mg/L; he was determined to be an UM of the drug (Koren et al., 2006). A 3 year-old-child who died after an accidental oral overdose had a postmortem serum codeine and morphine levels of 0.49 and 0.15 mg/L respectively (Ferreiros et al.m 2008). A 6 year-old-girl who died after being given 75 mg of the drug orally over 12 hours had a postmortem femoral blood codeine and morphine levels of 1.3 and 0.03 mg/L respectively (Martz and Litwin, 2011).

Conclusion

It is essential the clinician, most especially the pediatrician, and forensic pathologist, have a thorough understanding of polymorphic drug metabolism, as well as the effects of ethnicity, age and sex on metabolism, especially when it comes to commonly prescribed drugs, such as codeine. Considering what is understood of polymorphic drug metabolism and the effects of ethnicity, age and sex regarding codeine containing drugs, clinicians should use the lowest effective dose for the shortest time and inform their patients about the risks and signs. Regarding the recommendations of the advisory committee to the FDA restricting the use of codeine containing drugs in infants and children, it is clear from all available scientific information concerning polymorphic drug metabolism and the effects of ethnicity on such metabolism, the committees recommendation should be respected. If a mother is breast feeding and requires medication for cough suppression, the risk of infant exposure to codeine and morphine through breast milk should be weighed against the benefits of breastfeeding for both the mother and the baby. If a codeine containing product is used, the lowest dose should be prescribed for the shortest time to accomplish the desired clinical effect. Mothers using codeine should be informed about when to seek immediate medical care and how to identify the signs and symptoms of neonatal toxicity, such as drowsiness or sedation, difficult breastfeeding, breathing difficulties, and decreased tone, in their baby.