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Codeine-related deaths: The role of pharmacogenetics and drug interactions

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ABSTRACT

The objective of this study was to assess the relationship between genetic polymorphisms and drug interactions on codeine and morphine concentrations in codeine-related deaths (CRD). All CRD in Ontario, Canada between 2006 and 2008 were identified. Post-mortem blood was analyzed for 22 polymorphisms in 5 genes involved in codeine metabolism and response. Sixty-eight CRD were included in this study. The morphine-to-codeine ratio was significantly correlated with the presence of a CYP2D6 inhibitor at varying potencies ($p = 0.0011$). The presence of other central nervous system (CNS) depressants (i.e. benzodiazepines, hypnotics, and/or alcohol) was significantly associated with lower codeine concentration as compared to CRD in which other CNS depressants were not detected ($p = 0.0002$). Individuals who carried the *ABCB1 1236T* variant had significantly lower morphine concentrations ($p = 0.004$). In this population of individuals whose cause of death was related to codeine, drug interactions and genetic polymorphisms were significantly associated with post-mortem codeine and morphine concentrations.

1. Introduction

Over the last decade, the annual number of emergency department (ED) visits attributed to drug misuse or abuse has been steadily increasing. A report from the Drug Abuse Warning Network (DAWN) in 2011 estimated that over 1.2 million ED visits involved non-medical use of prescription medications in the United States. Twenty-nine percent of these visits were associated with non-medical use of prescription opioids [1]. Overall, the number of medical emergencies involving non-medical use of opioids rose 183% from 2004 to 2011 in the United States [1]. Coincident with the increased hospitalization involving prescribed opioids is a dramatic increase in the number of opioid-related deaths [2,3]. The majority of these opioid-related deaths also involved at least one non-opioid central nervous system (CNS) depressant [3]. Concomitant use of opioids with benzodiazepines, hypnotics, and/or alcohol, may enhance the depressive effects of opioids on respiratory drive [4–6].

Despite the known risks associated with concurrent use of opioids with non-opioid CNS depressants, it is difficult to document the toxicological effect of these drug interactions in human studies. In the absence of controlled-clinical trial data, toxicological data from post-mortem investigations are valuable, as they assist in the interpretation of drug concentrations in deaths involving opioid intoxication.

Codeine is a weak analgesic widely used in the management of mild-to-moderate pain. Morphine, the product of codeine *O*-demethylation by the highly polymorphic enzyme cytochrome P450 2D6 (CYP2D6), is the metabolite primarily responsible for the analgesic effect of codeine. The amount of morphine formed from codeine is highly variable, ranging from 0 to up to 75% [7] of total

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codeine metabolism. Originally, CYP2D6 enzymatic activity was determined by the urinary ratio of a specific CYP2D6 substrate to its *O*-demethylated metabolite. Subsequently, genotyping methods have segregated the population into four phenotypes: poor metabolizer (PM), intermediate metabolizer (IM), extensive metabolizer (EM), and ultra-rapid metabolizer (UM). It has been determined that PMs produce very limited morphine after codeine administration and experience inadequate pain relief [8,9]. On the other hand, UMs are at risk of experiencing opioid intoxication as a result of increased morphine production [7,10]. However, *CYP2D6* genotype does not fully predict phenotype. In particular, concomitant use of a CYP2D6 inhibitor may mimic a PM phenotype, leading to discordance between genotype-to-phenotype predictions. In addition to *CYP2D6*, previous studies by our group and others suggest that polymorphisms in the *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT* may be associated with both the anti-nociceptive and adverse effects of codeine and morphine [7,10–14].

The objective of the present study was to assess the relationship between genetic polymorphisms, drug interactions, and post-mortem morphine and codeine concentrations in codeine-related deaths (CRD).

2. Materials and methods

This study was approved by the Office of the Chief Coroner of Ontario (OCCO) and the Research Ethics Board of the Hospital for Sick Children in Toronto, Canada. In accordance with Ontario's *Coroners Act*, all sudden and unexpected deaths, and/or unnatural deaths must be reported to the OCCO. Coroner's death investigations involve classification of the medical cause of death. It also involves determination of the manner of death according to five categories: accident, homicide, natural, undetermined, and suicide. When necessary, a post-mortem examination that usually includes detailed toxicological testing is performed. Typical toxicological testing begins with screening for licit and illicit drugs by immunoassay and gas chromatography mass spectrometry (GC–MS), and screening for volatiles by headspace GC. This is followed by confirmation and quantitation by GC–MS or liquid chromatography (LC)–MS/MS, as required.

In a previous publication, population characteristics and descriptors associated with opioid-related fatalities in Ontario between 2006 and 2008 were described [15]. In this study, all drug-related deaths in which codeine was identified as a contributing factor to the fatality by the coroner were isolated. These deaths were attributed to codeine ingested alone or in combination with other pharmaceutical substances; deaths in which other circumstantial factors could have on their own resulted in fatality (i.e. homicide, external injuries, motor vehicle collisions, and disease) were not included. From this point, CRDs in which codeine, and its metabolite, morphine, were screened and quantified in femoral blood during the toxicological analysis were retrieved and reviewed. Using liquid chromatography tandem mass spectrometry, the limit of detection (LOD) for codeine and morphine was 32 ng/mL and 15 ng/mL, respectively. The limit of quantification (LOQ) for codeine and morphine was 63 ng/mL and 32 ng/mL, respectively. The data collected in this study were coded and analyzed anonymously. No personal identifiers were collected.

CRDs were excluded from the study if (i) the use of heroin was suspected (based on detection of 6-monoacetylmorphine (6-MAM) in biological fluids, and/or the death scene investigation indicated that heroin may have been used), (ii) morphine was suspected (based on the presence of morphine at the death scene investigation and/or a morphine prescription for the deceased), (iii) there was an indication of codeine misuse (i.e. inappropriate route of codeine administration), (iv) the manner of death was undetermined (v) femoral blood codeine and morphine concentrations were below the LOQ, and/or (vi) samples were of insufficient quality or quantity for genotype analysis.

In this study, a drug interaction was defined as concurrent detection of codeine and a CYP2D6 inhibitor [16,17], and/or the concurrent detection of a CNS depressant as part of toxicological analysis. The detected CYP2D6 inhibitors were divided into two groups according to their inhibitory potency [18]. Strong inhibitors consisted of bupropion, fluoxetine, and paroxetine. Weak inhibitors consisted of citalopram, diphenhydramine, and sertraline. Furthermore, CNS depressants consisted of benzodiazepines (e.g., alprazolam, clobazam, clonazepam, diazepam, lorazepam, oxazepam, and temazepam), hypnotics (e.g., zopiclone), ethyl alcohol, and/or other opioids (e.g., fentanyl, hydrocodone, hydromorphone, meperidine and oxycodone).

2.1. Genotyping

One milliliter of blood was collected from each CRD and used for genotype analysis. Genomic DNA was extracted from blood using the QiaSymphony DNA purification system (Qiagen, Toronto, Ontario, Canada) according to the manufacturer's protocol. Polymorphisms in *CYP2D6* were assessed using a modification of a previously described protocol based on multiplex single-base extension reaction (SNaPshot™; Applied Biosystems, Foster City, CA, USA) [14] for variants **2, *3, *4, *6, *7, *8, *9, *10, *12, *14, *17, *29, *41* and whole gene deletion (**5*) and duplications. Variants not carrying any detected mutations were classified as **1*. CYP2D6 activity score assignments were based on the method described by Gaedigk et al. [28].

Other genetic polymorphisms in the codeine and morphine pathway were also investigated. Morphine is a substrate of the efflux transporter P-glycoprotein, which is encoded by the *ABCB1* gene. Three variants in *ABCB1* were targeted for genotyping (rs1128503, rs2032582, rs1045642). *ABCB1* haplotypes using these three markers (*C1236T*, *G2677T*, and *C3435T*, respectively) have been shown to have an effect on the overall function of P-glycoprotein by means of conformational changes to the protein structure [19]. The UDP-glucuronosyltransferase *(UGT) 2B7* gene was also of interest as it largely mediates morphine glucuronidation into both active and inactive metabolites [20]. The *UGT2B7*2* variant (rs62298861) is associated with increased UGT2B7 enzyme activity [21]. We also targeted the *OPRM1* gene which encodes the mu opioid receptor. The *OPRM1 A118G* variant (rs1799971) has been associated with reduced expression at the brain [22]. Lastly, we targeted the catechol *O*-methyltransferase gene, (*COMT*), which metabolizes catecholamines and has been associated with pain sensitivity [23]. *COMT C389T*, *C611G/T*, and *G675A* (rs4633, rs4818, rs4680, respectively) are suggested to contribute to pain perception and influence morphine-related side effects [24–27]. Genotyping for *ABCB1* (rs1128503, rs2032582, rs1045642), UGT2B7 (rs62298861), *OPRM1* (rs1799971), and *COMT* (rs4633, rs4818, rs4680) were all conducted using TaqMan® Genotyping assays (Life Technologies/Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommendations as described previously [14]. Genotyping for the *UGT2B7*2* variant was conducted by RFLP as described previously [14].

2.2. Statistical analysis

Statistical analysis was performed using SPSS (IBM, version 20, Somers, NY). Characteristics of CRDs in which the manner of death was deemed to be accidental or suicide were compared by the Chi-square test or Fischer's exact test for categorical variables and Student's *t*-test and one-way ANOVA for continuous variables. The M/C ratio, codeine concentration, and morphine concentration among the genetic polymorphisms of interest were analyzed using