Identification of Hydrocodone in Human Urine Following Controlled Codeine Administration

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Abstract

Allegations of illicit hydrocodone use have been made against individuals who were taking physician-prescribed oral codeine but denied hydrocodone use. Drug detection was based on positive urine opiate immunoassay results with subsequent confirmation of hydrocodone by gas chromatography–mass spectrometry (GC–MS). In these cases, low concentrations of hydrocodone (approximately 100 ng/mL) were detected in urine specimens containing high concentrations of codeine (> 5000 ng/mL). Although hydrocodone has been reported to be a minor metabolite of codeine in humans, there has been little study of this unusual metabolic pathway.

We investigated the occurrence of hydrocodone excretion in urine specimens of subjects who were administered codeine. In a controlled study, two African-American and three Caucasian male subjects were orally administered 60 mg/70 kg/day and 120 mg/70 kg/day of codeine sulfate on separate days. Urine specimens were collected prior to and for approximately 30–40 h following drug administration. In a second case study, a postoperative patient self-administered 960 mg/day (240 mg four times per day) of physician-prescribed oral codeine phosphate, and urine specimens were collected on the third day of the dosing regimen. Samples from both studies were extracted on copolymeric solid-phase columns and analyzed by GC–MS. In the controlled study, codeine was detected in the first post-drug-administration specimen from all subjects. Peak concentrations appeared at 2–5 h and ranged from 1475 to 61,695 ng/mL. Codeine was detected at concentrations above the 10-ng/mL limit of quantitation for the assay throughout the 40-h collection period. Hydrocodone was initially detected at 6–11 h following codeine administration and peaked at 10–18 h (32–135 ng/mL). Detection times for hydrocodone following oral codeine administration ranged from 6 h to the end of the collection period. Confirmation of hydrocodone in a urine specimen was always accompanied by codeine detection. Codeine and hydrocodone were detected in all specimens collected from the postoperative patient, and concentrations ranged from 2099 to 4020 and 47 to 129 ng/mL, respectively. Analyses of the codeine formulations administered to subjects revealed no hydrocodone present at the limit of detection of the assay (10 ng/mL). These data confirm that hydrocodone can be produced as a minor metabolite of codeine in humans and may be excreted in urine at concentrations as high as 11% of parent drug concentration. Consequently, the detection of minor amounts of hydrocodone in urine containing high concentrations of codeine should not be interpreted as evidence of hydrocodone abuse.

Introduction

Our laboratory has received inquiries about the potential for hydrocodone formation following codeine ingestion in individuals who denied any illicit hydrocodone use but tested positive for opiates in a forensic urine drug test. Forensic evidence was based on gas chromatographic–mass spectrometric (GC–MS) confirmation of low concentrations of hydrocodone (approximately 100 ng/mL) in urine specimens containing high concentrations of codeine (> 5000 ng/mL). Documented codeine prescriptions existed for these individuals for licit analgesic or antitussive regimens, and their individual histories were not indicative of opiate abuse. The frequency of inquiries and magnitude of resulting punitive consequences for these individuals prompted us to investigate the occurrence of hydrocodone in urine of patients who were administered codeine.

The 6-keto-opioid hydrocodone (dihydrocodeinone) is most commonly prescribed for its antitussive and analgesic effects. It is approximately six times more potent than codeine, equipotent to morphine, and has an addiction liability similar to morphine (1,2). Significant cross-reactivity for hydrocodone has been demonstrated for several opiate screening assays resulting in the potential for positive urinalysis screening (1,3). Historically, forensic confirmation of hydrocodone in urine specimens without substantiation of a valid prescription has been interpreted as evidence of illicit hydrocodone use.

The semisynthetic opiate codeine produces analgesic and antitussive effects that are primarily centrally mediated (4,5). The mechanisms of these effects are not completely understood, but CNS effects have been attributed to codeine as well as the metabolite morphine and minor metabolite norcodeine. It is commonly prescribed to patients experiencing mild to moderately severe pain and those with pathological cough symptoms. Its therapeutic utility also includes sedation and treatment of acute pulmonary edema and diarrhea. Codeine has significant abuse liability and contributes to emergency room mentions for narcotics in the

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United States. It is routinely included in screening panels and confirmation tests for drugs of abuse. The phosphate (and less commonly sulfate) salt is formulated alone or in combination with peripherally acting analgesics and in cough suppressant elixirs. The recommended analgesic dosage range for adults is 15–60 mg with a 360 mg/day recommended maximum (6). Unlike morphine, codeine is absorbed well orally with approximately 80–90% excreted as free and conjugated parent drug (of which 10% is free base and 90% is codeine-6-glucuronide). The remainder is excreted primarily as the glucuronide conjugates of morphine and norcodeine (7,8). Previous clinical half-life estimates for free codeine in urine range from 2.7 to 12.2 h (7,9). Codeine is excreted at a higher rate than creatinine implying both glomerular filtration and active tubular secretion (8). In controlled studies, the pattern of urinary excretion is independent of dose, route of administration, or degree of addiction or tolerance, and the maximum metabolism and elimination rate has been estimated to be approximately 30 mg/h (8). Therefore, implementation of maximal dosage regimens can give rise to codeine accumulation in blood (6,8).

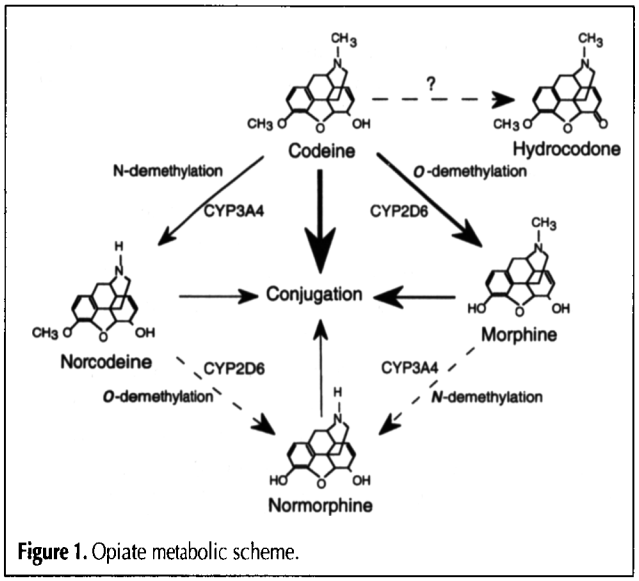

Figure 1. Opiate metabolic scheme.

Codeine metabolism occurs primarily in the liver where it can be *O*-demethylated to morphine with subsequent *N*-demethylation to normorphine or *N*-demethylated to norcodeine with subsequent *O*-demethylation to normorphine (Figure 1). The P450 liver enzymes responsible for *O*- and *N*-demethylation are CYP2D6 and CYP3A4, respectively (10). Minor oxidative pathways for formation of the ketone derivatives of other drugs have been demonstrated in human liver microsomes and cytosol. It has been postulated that the aldo-keto reductase family of enzymes mediate the formation of E- and Z-10-oxonortriptyline from 10-hydroxylated nortriptyline metabolites (11). The existence of minor oxidative pathways has also been documented for morphine and codeine. The conversion of morphine to hydromorphone has been reported in other mammalian species, and in one clinical study, Cone et al. (12) postulated that the formation of minor amounts of hydrocodone and several other unique metabolites following controlled administration of oral codeine to healthy, noncompromised patients could occur by the same mechanism.

This report is a composite of data collected from two clinical studies to investigate the unique oxidative metabolism of codeine to hydrocodone. The first was a controlled codeine administration study in humans. The second was a postoperative outpatient clinical case study.

Materials and Methods

Chemicals and reagents

Codeine sulfate for human administration in the controlled clinical study was obtained from Roxane Laboratories (Columbus, OH) and prepared in lactose capsules (Amend Drug and Chemical Co., Inc., Irvington, NJ) for oral administration. Codeine phosphate administered to the postoperative patient was physician-prescribed and acquired from a commercial pharmacy. Chemicals included in urinalysis were obtained from the following sources: codeine phosphate and hydrocodone bitartrate from Sigma

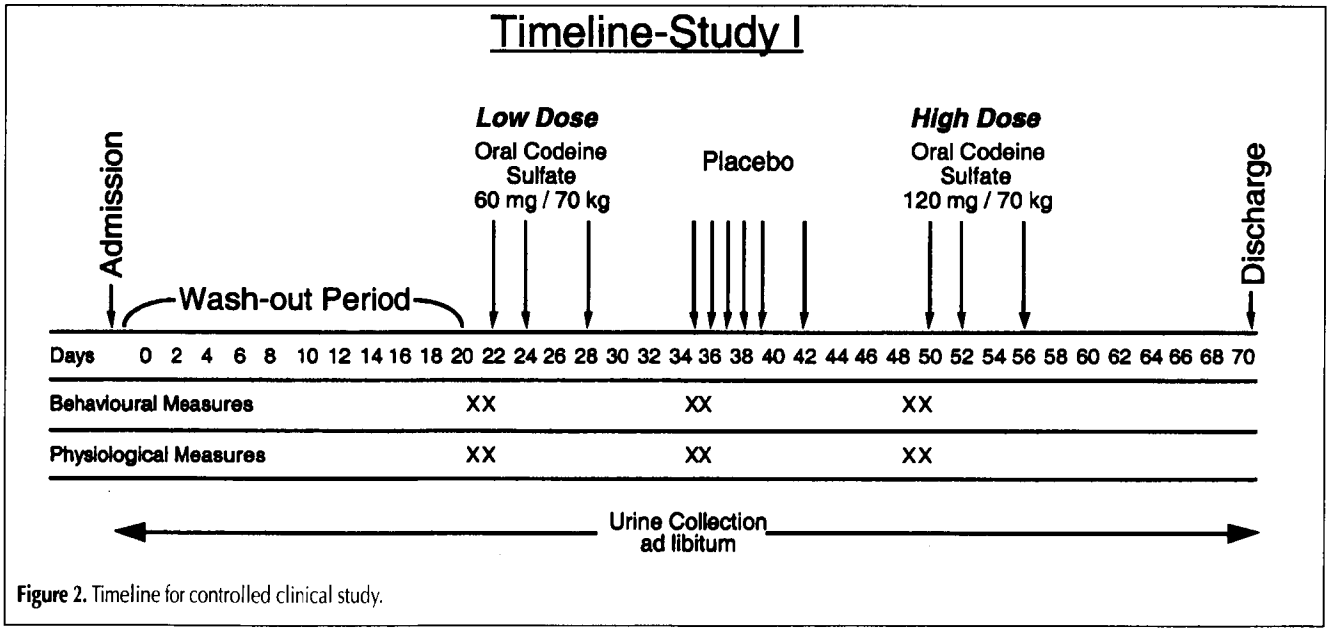

Figure 2. Timeline for controlled clinical study.

Chemical Co. (St Louis, MO) and [$^2H_3$]-codeine from Radian (Austin, TX). Solid-phase extraction (SPE) columns (Clean Screen DAU, 200 mg-10 mL, United Chemical Technologies, Inc., Bristol, PA). Methanol, methylene chloride, 2-propanol, and acetonitrile were HPLC-grade chemicals. All other chemicals were reagent grade.

Research subjects

In the controlled clinical study, two African-American and three Caucasian males participated in a 10-week NIDA Institutional Review Board approved inpatient study conducted at the Intramural Research Program, NIDA. All subjects had a history of opioid abuse, provided written informed consent, and were paid for their participation. Physical and psychological screenings were performed to ensure subjects were healthy. None of the subjects were determined to be physically dependent on drugs or medications, with the possible exception of nicotine and caffeine. During the study, subjects resided on the secured research unit at the Intramural Research Program, National Institute on Drug Abuse. Daily urine drug testing was conducted to monitor subjects' compliance with study guidelines and to ensure that there were no self-administrations of drugs and over-the-counter medications. Urine screening was conducted by the National Center for Forensic Sciences (Baltimore, MD). Screening for opioids was performed by immunoassay with EMIT II reagents (Syva, San Jose, CA). Each urine void was collected over the entire 10-week study in polypropylene bottles and stored at –30°C until analysis by GC–MS.

In the case study, one Caucasian male self-administered oral codeine phosphate as part of a therapeutic outpatient regimen. The patient was healthy and not dependent on drugs. Three days after initiation of the regimen, urine specimens were collected in polypropylene bottles and stored at –30°C until analysis by GC–MS.

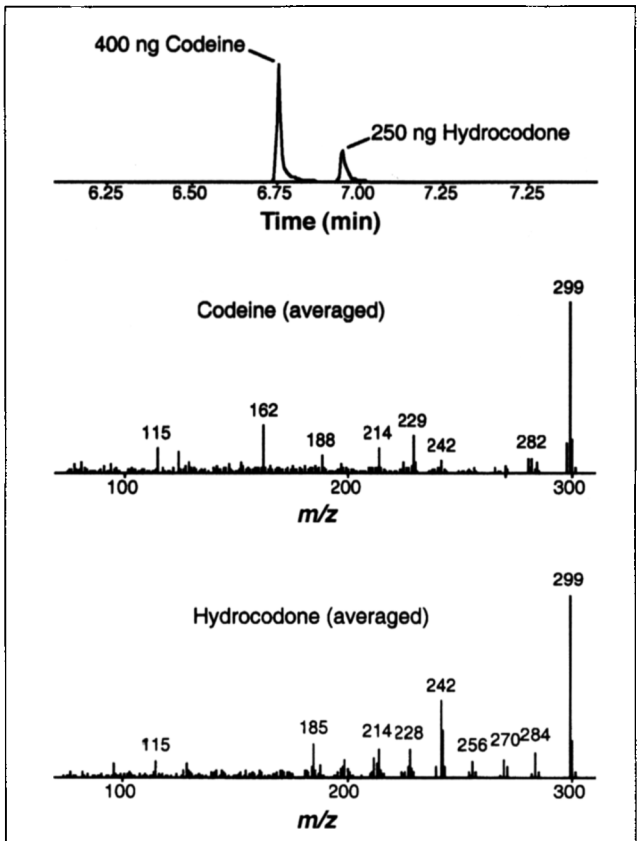

Figure 3. Chromatographic and mass spectral characterization of underivatized codeine and hydrocodone (unextracted 400 ng codeine and 250 ng hydrocodone standards).

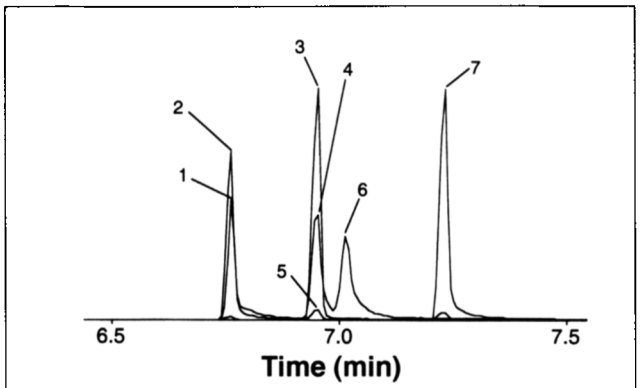

Figure 4. Target ion chromatogram of 2000 ng of the following analytes: peak 1, norcodeine (*m/z* 285); peak 2, codeine (*m/z* 299); peak 3, hydrocodone (*m/z* 299); peak 4, morphine (*m/z* 285); peak 5, normorphine (*m/z* 271); peak 6, hydromorphone (*m/z* 285); and peak 7, 6-acetylmorphine (*m/z* 327)

Study protocol

Figure 2 illustrates the overall study timeline for the controlled study. Participants began the study within one week of admission. During the entire study, subjects' vital signs were checked daily and routine blood tests (CHEM Profile 2) were performed every two weeks to monitor subjects' health. Subjects underwent an initial three-week washout or clearance phase to permit drug to be eliminated from biological tissues and matrices. Urine specimens were collected once a week during the wash-out phase, and all voids were collected following the initiation of drug administration. In week 4 (low-dose week), subjects received 60 mg of codeine sulfate/70 kg (25.78 codeine base mg equivalents/70 kg) by the oral route on Tuesday, Thursday, and the following Monday. Doses of codeine were increased to 120 mg/70 kg (51.56 base mg equivalents/70 kg) in week 8 (high-dose week). The last urine specimen collected prior to drug administration and all voids collected for 30–40 h following the third administration of each dose were analyzed. Urine specimens from all subjects were negative for codeine and metabolites prior to initiation of drug administration.

In the second study, the patient orally self-administered 960 mg (240 mg four times per day) of codeine phosphate (approximately 370 codeine base mg equivalents/70 kg) daily as part of a postoperative analgesic regimen. All urine specimens were collected on the third day of the regimen.

Analysis of urine

Codeine and hydrocodone were extracted from unhydrolyzed urine specimens according to a previously described method (13). Briefly, an aliquot of aqueous internal standard solution (100 ng codeine-d_3) was added to 1 mL of urine in a glass tube. Excess (3:1, v/v) sodium acetate buffer (pH 4.0, 0.5M) was added, and samples were allowed to sit at room temperature for approximately 10 min. Following centrifugation (5 min at 1500 RPM),

the supernatant was decanted onto previously conditioned copolymeric SPE columns. Columns were then subjected to a series of washes, and analytes were eluted with methylene chloride, 2-propanol, and concentrated ammonium hydroxide (80:20:2, v/v/v). Samples were placed in a water bath at approximately 40°C, and solvent was evaporated under a stream of nitrogen. Following reconstitution with 20 µL of acetonitrile, 1 µL of underivatized sample was injected into the GC–MS for analysis.

Spectral and chromatographic characterization was performed in full-scan mode. Employing the referenced GC–MS conditions, codeine and hydrocodone eluted at approximately 6.75 and 6.90 min, respectively. Base peaks for the analytes were their respective molecular ions (*m/z* 302 for codeine-d_3 and 299 for codeine and hydrocodone) which were then employed as quantitation or target ions in selected ion monitoring mode for quantitative analysis. Mass-to-charge fragments 162, 229, and 285 were employed as confirming or qualifying ions for codeine, and confirming ions for hydrocodone included *m/z* 242, 185, and 214 (Figure 3). Characterization of other opiate analytes was also performed to ensure that coelution of analogues with common mass-to-charge ratio ion fragments did not occur. Additional analytes characterized were 6-acetylmorphine, morphine, norcodeine, normorphine, and hydromorphone (Figure 4).

Duplicate calibration curves for codeine and hydrocodone were constructed with drug-free urine. Linear regression coefficients (r^2) were ≥ 0.99 for both analytes over a concentration range of 10–1000 ng/mL. The limits of detection (LOD) and quantitation (LOQ) for codeine and hydrocodone were 10 ng/mL. A serial dilution of analytical controls with concentrations ranging from 1 to 100 ng/mL was constructed in quadruplicate and analyzed. LOD was defined as the concentration at which the analyte quantitating ion signal-to-noise ratio (determined by peak height) was > 3:1 and 75% had ion ratios within ± 20% of those observed for 100 ng calibration standards analyzed in the same batch. The LOQ definition included LOD criteria plus the requirement that 75% of the controls at that concentration quantitate within ± 20% of the target concentration. Duplicate analytical codeine controls at 500 ng of codeine base/mL of urine were analyzed with each batch and were required to quantitate within ± 20% of the target concentration. Clinical controls composed of the codeine formulations from both studies at codeine base equivalent concentrations of approximately 5000 ng (250 ng of base on-column) were also analyzed for codeine and hydrocodone.

Instrumentation

Quantitative analysis of urine was performed on a Hewlett-Packard 5890A GC outfitted with an autosampler (HP 7673A) and interfaced with a Hewlett-Packard 5972 mass selective detector in electron impact mode. The capillary inlet system was set in the splitless mode. The GC column employed in analysis was a Phenomenex ZB1 fused-silica capillary column (15 m × 0.25-mm i.d., 0.10-µm film thickness), and analysis was performed according to a previously published GC–MS procedure (14).

Results

Purity of analytical and pharmaceutical codeine preparations

Prior to the analysis of clinical urine samples, control solutions

Table I. Urine Concentrations of Codeine and Hydrocodone after Oral Codeine Sulfate Administration

Subject A			Subject B			Subject C			Subject E			Subject F		
Time (h)*	Codeine (ng/mL)	Hydrocodone (ng/mL)	Time (h)*	Codeine (ng/mL)	Hydrocodone (ng/mL)	Time (h)*	Codeine (ng/mL)	Hydrocodone (ng/mL)	Time (h)*	Codeine (ng/mL)	Hydrocodone (ng/mL)	Time (h)*	Codeine (ng/mL)	Hydrocodone (ng/mL)
Low dose (60 mg/70 kg)														
-12.42	77	0	-4.25	0	0	-3.00	0	0	-0.92	267	0	-1.75	394	0
1.58	758	0	2.33	17092	0	2.50	15160	0	2.25	27273	0	1.42	16318	0
2.66	1003	0	7.25	4612	10	3.67	13974	29	4.50	12779	0	18.17	14509	0
5.33	1329	23	12.25	2469	16	10.00	9607	32	8.83	3689	42	23.02	10811	20
7.42	1476	40	20.92	1578	0	28.50	893	18	12.17	6989	0	14.67	2582	14
23.66	1022	80	26.75	488	0	14.92	4746	0	47.00	1970	11			
27.66	201	49	20.08	421	0	48.17	409	11						
31.75	175	50	26.17	293	0									
33.33	128	23												
34.53	105	19												
40.53	88	15												
High dose (120 mg/70 kg)														
-1.50	0	0	-4.25	114	0	-1.48	0	0	-4.00	4550	0	-0.92	97	0
1.42	1580	0	2.58	34827	0	2.53	26851	14	2.25	12462	11	0.83	5011	0
4.50	3666	0	8.33	15001	21	6.52	32177	51	4.00	14557	0	1.55	61695	23
9.00	2918	85	12.67	5439	16	8.83	31659	26	9.25	4401	0	17.00	40673	0
17.50	1222	135	19.92	2227	22	11.52	12130	41	11.25	3700	14	18.27	24612	0
20.50	493	62	28.17	575	7	14.67	11389	14	19.50	518	6	21.53	30538	0
26.83	201	47	17.75	4944	39	25.58	986	31	24.18	11060	0			

* Collection time from last administration of each dose.

of the analytical and pharmaceutical codeine standards were constructed in drug-free urine. Duplicate codeine 1000- and 2000-ng/mL analytical controls and samples from both pharmaceutical formulations equivalent to approximately 5000 ng codeine base/mL urine (approximately 250 ng base on column) were analyzed, and no hydrocodone was detected. Duplicate codeine 500-ng/mL analytical controls were also analyzed with each sample batch, and hydrocodone was not detected. The lack of hydrocodone detection in these control samples excludes the possibility of detectable production of hydrocodone as an artifact in the assay or as an impurity in the analytical or pharmaceutical preparations. Because urine samples are commonly hydrolyzed before GC–MS analysis in forensic laboratories, we also subjected 5000-ng codeine and morphine controls prepared in drug-free urine to acid hydrolysis. Samples were hydrolyzed by addition of concentrated HCl to a final concentration of 10% (v/v). Samples were then incubated at 80°C for 2 h. Sodium acetate buffer (pH 4.0, 2.0M) was added to adjust the pH of samples to approximately 4.0 followed by SPE and GC–MS analysis. Absence of hydrocodone detection following hydrolysis also excludes potential for formation of hydrocodone as a hydrolytic artifact.

Codeine and hydrocodone disposition in urine—study I

Urine specimens were collected ad libitum for 30–40 h following codeine administration. Table I lists the specimen collection times relative to dosing and urine codeine and hydrocodone concentrations. Codeine was detected in the first specimen collected following each drug administration. Peak codeine concentrations (C_{max}) occurred at 2–5 h and ranged from 1475 to 61,695 ng/mL. An intrasubject dose-concentration relationship for codeine excretion in urine was evident following oral administration of low- and high-dose regimens. However, intersubject C_{max} comparisons were highly variable. Initial hydrocodone detection occurred in specimens collected 6–11 h following drug administration. Hydrocodone concentrations peaked at 10–18 h, and concentrations ranged from 32 to 135 ng/mL. Both the observed initial detection and C_{max} for hydrocodone occurred later than that observed for codeine. Hydrocodone detection times generally extended to the last urine analyzed. A cursory comparison of the slopes of the terminal excretion curves for codeine and hydrocodone following all but one of the drug administration sessions implied a longer half-life for hydrocodone (the high dose session for Subject F had only one specimen positive for hydrocodone). At the time of peak codeine concentrations, hydrocodone concentrations ranged from 1 to 5% of parent drug concentrations. However, at the peak of hydrocodone excretion, hydrocodone concentrations ranged from < 1 to 11% of codeine.

Table II. Urine Concentrations of Codeine and Hydrocodone after Oral Codeine Phosphate Administration to One Postoperative Patient

Time (h)*	Codeine (ng/mL)	Hydrocodone (ng/mL)
56.50	2252	129
62.33	2889	77
64.25	3219	69
72.00	3301	47
76.00	2100	53
80.48	1872	72
85.00	4020	66
90.15	2890	62

* Time from initiation of 960 mg/day (240 mg four times per day) oral codeine phosphate regimen.

Codeine and hydrocodone disposition in urine—study II

Sequential urine specimens were collected from a healthy Caucasian male on the third day of a postoperative oral codeine phosphate regimen. Codeine and hydrocodone were detected in all specimens analyzed at concentrations ranging from 2099 to 4020 and from 47 to 310 ng/mL, respectively. Table II lists the specimen collection times and analyte concentrations in urine. Hydrocodone/codeine ratios ranged from 2 to 6% for all specimens analyzed.

The reader should be aware that observed hydrocodone to codeine ratios from both studies resulted from the analysis of unhydrolyzed urine specimens and were therefore relative to free codeine concentrations. A comparison of hydrocodone to total codeine concentrations in urine following codeine administration could result in altered ratios.

Discussion

Codeine is widely prescribed as an analgesic and antitussive and is supplied alone and in various combinations with other substances such as acetaminophen, caffeine, acetylsalicylic acid, butalbital, and guaifenesin. Codeine dosages range from 8 to 60 mg. The recommended oral doses used routinely in treatment of chronic pain for around-the-clock analgesia are approximately 100 mg every 4 h. Dosages above 1.5 mg/kg of body weight/day are not recommended because of increased toxicity. At the highest recommended dose, individuals could ingest up to 600 mg of codeine per 24 h. An acute lethal dose of codeine for a nontolerant adult has been estimated at 0.5–1.0 g (15). Urine drug testing of individuals taking prescribed codeine frequently involves measurement of codeine and morphine concentrations. Individuals who document licit ingestion of codeine are usually not suspected of illicit abuse of opiates. However, the presence of hydrocodone, even at low concentrations when compared to codeine, has been construed as evidence of illicit hydrocodone use. Current findings do not support these conclusions and indicate that low concentrations of hydrocodone may be excreted in urine of individuals who have ingested codeine. The observed 6–11 h delay in the detection of hydrocodone and the longer elimination half-life for hydrocodone that these data imply were suggestive of metabolic formation rather than excretion of hydrocodone as an impurity of the codeine preparation. In addition, analysis of the codeine administered in both clinical studies revealed no detectable amounts of hydrocodone as an impurity in the two dosage forms. Consequently, the origin of the hydrocodone identified in urine appears to be biotransformation of codeine.

Oxidative metabolism of codeine leads primarily to the formation of norcodeine and morphine. Subsequent conjugation of

codeine and these metabolites leads to excretion, primarily in urine. The sum total of free and conjugated codeine and other metabolites account for approximately 95% of a single dose (16). The minor metabolite, hydrocodone, appears to form as a result of poorly defined metabolic pathways. Morphine-6-dehydrogenase has been reported to transform morphine to morphinone in guinea pigs (17). It is possible that morphinone, having an α,β-unsaturated ketone group, could be enzymatically reduced to hydromorphone. A similar pathway could also exist for the formation of hydrocodone from codeine. Another possibility could be the oxidation of codeine by the aldo-keto reductase enzyme family as has been reported for the 10-hydroxy metabolites of amitriptyline and nortriptyline (11). Finally, enzymatic rearrangement of codeine to hydrocodone may occur. However, none of these pathways have been fully characterized, and the mechanism of hydrocodone formation remains uncertain.

Formation of hydrocodone from codeine could lead to the production of additional hydrocodone-related metabolites. Indeed, Cone et al. (12) reported detection of 6-keto-reduction products (6α-hydrocodol and 6β-hydrocodol) and *N*-demethylation products (norhydrocodone) at low concentrations relative to codeine in urine of humans and guinea pigs following codeine administration.

Clearly, source differentiation of opiates can be problematic. The identification of hydrocodone in urine in low concentrations relative to other opiates such as codeine should not be taken as confirmation of illicit hydrocodone use. The presence of impurities in opiate preparations as well as the formation of unusual metabolites of morphine and codeine in low concentrations should be considered in the interpretation of these cases. It would seem likely, based on current knowledge, that numerous minor opiate metabolites are produced through as yet uncharacterized metabolic pathways. These data confirm the existence of one or more minor pathways for codeine metabolism that could result in urinary elimination of detectable concentrations of hydrocodone in humans following oral codeine administration.

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