Urine Drug Testing of Chronic Pain Patients. II. Prevalence Patterns of Prescription Opiates and Metabolites

Rebecca Heltsley[1], Anne Zichterman[1], David L. Black[1], Beverly Cawthon[1], Tim Robert[1], Frank Moser[1], Yale H. Caplan[2], and **Edward J. Cone[3,*]**

[1]Aegis Sciences Corporation, 515 Great Circle Road, Nashville, Tennessee; [2]National Scientific Services, 3411 Phillips Drive, Baltimore, Maryland; and [3]Johns Hopkins School of Medicine, Baltimore, Maryland

Abstract

This study of 20,089 urine specimens from chronic pain patients provided a unique opportunity to evaluate the prevalence of prescription opiates and metabolites, assess the usefulness of inclusion of normetabolites in the test panel, and compare opiate and oxycodone screening results to liquid chromatography with tandem mass spectrometry (LC–MS–MS) results. All specimens were screened by an opiate [enzyme-linked immunosorbent assay (ELISA), 100 ng/mL] and oxycodone assay [ELISA, 100 ng/mL or enzyme immunoassay (EIA), 50 ng/mL] and simultaneously tested by LC–MS–MS [limit of quantitation (LOQ) = 50 ng/mL] for 10 opiate analytes (codeine, norcodeine, morphine, hydrocodone, dihydrocodeine, norhydrocodone, hydromorphone, oxycodone, noroxycodone, and oxymorphone). Approximately two-thirds of the specimens were positive for one or more opiate analytes. The number of analytes detected in each specimen varied from 1 to 8 with 3 (34.8%) being most prevalent. Hydrocodone and oxycodone (in combination with metabolites) were most prevalent followed by morphine. Norcodeine was only infrequently detected whereas the prevalence of norhydrocodone and noroxycodone was approximately equal to the prevalence of the parent drug. A substantial number of specimens were identified that contained norhydrocodone (*n* = 943) or noroxycodone (*n* = 702) but not the parent drug, thereby establishing their interpretative value as biomarkers of parent drug use. Comparison of the two oxycodone screening assays revealed that the oxycodone ELISA had broader cross-reactivity with opiate analytes, and the oxycodone EIA was more specific for oxycodone. Specimens containing only norhydrocodone were best detected with the opiate ELISA whereas noroxycodone (only) specimens were best detected by the oxycodone EIA.

Introduction

Opioids are the mainstay of pharmacotherapy in patients suffering from acute and chronic pain. Opioid drugs are generally safe and efficacious when used as prescribed but also have powerful reinforcing properties and high abuse potential for some individuals. Clinicians who prescribe opioids are concerned about the risks of under-treatment, over-prescription, and the possibility of opioid addiction (1). Drug testing of chronic pain patients maintained on opioids provides objective information about recent use of prescribed and unauthorized drugs and illicit drugs. For patients prescribed one or more of the structurally related "opiate" drugs, interpretation of their test results can be difficult because of the multiple metabolic pathways accorded these compounds in the human body prior to their excretion. Interpretation is especially difficult for those drugs that are biotransformed into other commercially available prescription opiates. For example, two of the most frequently prescribed analgesics, hydrocodone and oxycodone, undergo *O*-demethylation to hydromorphone and oxymorphone, respectively. In addition, hydrocodone undergoes 6-keto-reduction to dihydrocodeine (6-α-hydrocodol). These metabolites are also available commercially and are sold as prescription analgesics and as antitussives. Consequently, a

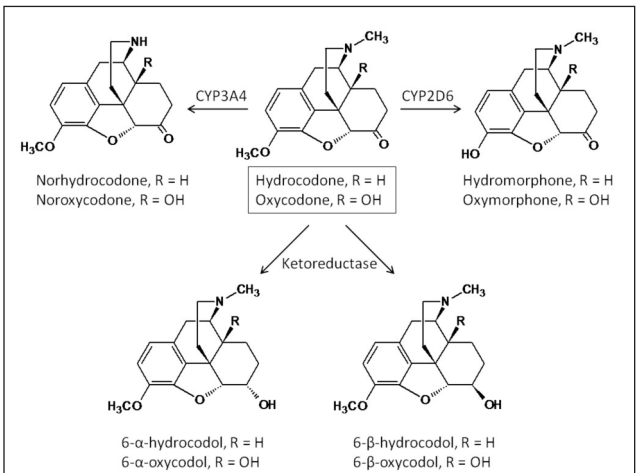

Figure 1. Metabolic pathways for the *N*-demethylation, *O*-demethylation, and 6-keto-reduction of hydrocodone and oxycodone.

* Author to whom correspondence should be addressed: Edward J. Cone, 441 Fairtree Drive, Severna Park, Maryland 21146. E-mail: Edward.cone@comcast.net.

positive test for the parent drug and/or its metabolites does not reveal whether the parent drug was the source of the "metabolite" or if multiple drugs were taken.

The primary Phase I enzymatic pathways by which hydrocodone and oxycodone are metabolized are illustrated in Figure 1. Hydrocodone and oxycodone are transformed by the enzyme cytochrome P450 2D6 (CYP2D6) to hydromorphone (2) and oxymorphone (3), respectively. Both metabolites have about a 30-fold higher affinity for the mu receptor than the parent drug (4), thus making them significantly more potent than the parent drug. Genetic polymorphisms of CYP2D6 leads to large interindividual differences in the formation and disposition of these metabolites. Individuals who have diminished CYP2D6 activity are known as poor metabolizers (PMs). These individuals produce and excrete very little *O*-demethylated metabolite in urine following hydrocodone or oxycodone administration (2). The remainder of the population have highly variable CYP2D6 activity, ranging as much as 1000-fold among individuals (5), and are divided into three groups: intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultrarapid metabolizers (UMs). The latter group, UMs, have alleles with repeat copies of the CYP2D6 gene, thus producing larger amounts of this enzyme. In addition to genetic differences, inhibition of CYP2D6 activity can occur when another drug binds with the enzyme and inhibits its action. For example, quinidine is a potent inhibitor of CYP2D6 activity; a single dose can convert EMs to PMs, thereby reducing or eliminating formation of the *O*-demethylated metabolite (5).

An alternate metabolic pathway via cytochrome P450 3A4 (CYP3A4) enzyme leads to *N*-demethylated metabolites of hydrocodone (6) and oxycodone (7). In vitro studies indicate that CYP3A5 also may contribute to *N*-demethylation of oxycodone (7). In contrast to *O*-demethylation, *N*-demethylation leads to a diminution in analgesic activity (8–10). The CYP3A4 enzyme is subject to many drug interactions, which may induce or inhibit the enzyme. Some common CYP3A4 inhibitors include ketoconazole, macrolide antibiotics (e.g., clarithromycin, erythromycin), antiretrovirals (e.g., ritonavir, delavirdine), antidepressants (e.g., fluoxetine, fluvoxamine), and calcium channel blockers (e.g., verapamil, diltiazem) (6,11). CYP3A4 may also be induced by rifampin (12), anticonvulsants (e.g., carbamazepine, phenytoin, primidone, phenobarbital), and the antiretrovirals efavirenz and nevirapine (11). Induction of CYP3A4 activity by rifampin was shown to be the cause of negative urine tests for oxycodone in a chronic pain patient treated with immediate and controlled-release oxycodone (13). Because of the absence of oxycodone in the patient's urine, he was suspected of non-adherence to prescribed regimen and possible drug diversion. Further testing of the patient's specimen revealed small amounts of oxymorphone and larger amounts of noroxycodone were present indicating the patient was drug compliant.

A third metabolic pathway for hydrocodone and oxycodone is 6-keto-reduction to the respective epimeric alcohols. Two orientations of the hydroxyl-group at C-6 can be produced by metabolic reduction leading to two distinct alcoholic compounds (14,15). The orientations of the 6-α- and 6-β-hydroxymetabolites are illustrated in Figure 1. Generally, these metabolites are considered equivalent to weaker analgesics than the parent compound (15)

Codeine undergoes both *O*-demethylation via CYP2D6 to morphine and *N*-demethylation via CYP3A4 to norcodeine (16). A minor metabolic pathway for codeine via an uncharacterized enzyme system(s) leads to the formation of hydrocodone (17). A similar minor metabolic pathway has been reported to occur for the conversion of morphine to hydromorphone (18,19).

Single dose studies of codeine (20,21), morphine (22), hydrocodone (23), hydromorphone (23), dihydrocodeine (24), oxycodone (23), and oxymorphone (23) have provided information on the general timecourse and excretion patterns of parent drug and *O*-demethylated metabolites in urine; however, little information is available regarding the excretion patterns of the normetabolites of these compounds. Further, little is known regarding how excretion patterns change in populations who ingest these drugs on a daily regimen.

This study is part of an ongoing series of research studies designed to improve testing methods for monitoring pain patients (1). The goal of this study was to characterize the prevalence of prescription opiate drugs and related *O*- and *N*-demethylated metabolites in a large population of chronic pain patients who were prescribed opiates on a daily basis. An earlier pilot study of 2654 specimens from pain patients revealed that monitoring for normetabolites reduced false-negative results by approximately 10% (25). In this study, a database comprised of 20,089 test results is described in which all specimens were tested simultaneously by immunoassay (IA) and mass spectrometry (MS). Specimens were screened for opiates and oxycodone by IA and confirmed for 10 opiate drugs and metabolites by liquid chromatography (LC)–tandem MS. Two secondary goals of the study were to assess the potential interpretative usefulness of norcodeine, norhydrocodone, and noroxycodone as biomarkers of codeine, hydrocodone, and oxycodone use, respectively, and to compare opiate and oxycodone screening results to confirmation results.

Experimental

Subjects and specimens

A total of 20,089 urine specimens from chronic pain patients were submitted to Aegis Sciences (Nashville, TN) over the period of August–December 2008 for drug testing. The specimens originated from 230 pain clinics in 24 states. During this period, screening assays for opiates and oxycodone were performed simultaneously with confirmation testing. The screening and confirmation data were assembled into a single database for evaluation of drug and metabolite patterns and comparison of screening to confirmation results. The protocol for this study was approved by the Essex Institutional Review Board (Lebanon, NJ).

Screening procedures

Screening for opiates was performed with Opiates Direct ELISA Kit (Immunalysis, Pomona, CA). The cutoff concentra-

tion for the assay was 100 ng/mL. According to the package insert (Version 05/2009), cross-reactivities for different opiate analytes were as follows: morphine (100%); codeine (200%); dihydrocodeine (85%); hydrocodone (93%); 6-acetylmorphine (83%); hydromorphone (81%); morphine-3-gulucuronide (62.5%); 6-acetylcodeine (41.7%); oxycodone (21%); oxymorphone (20%); norcodeine (8.3%); noroxycodone (< 0.25%); normorphine (3%); noroxymorphone (< 0.25%); and morphine-6-glucuronide (< 4%).

Two oxycodone screening assays were employed for testing of the 20,089 specimens. The first 7404 specimens (August 13, 2008 to October 4, 2008) were tested with Oxycodone Direct ELISA Kit (Immunalysis) at a cutoff concentration of 100 ng/mL. According to the package insert (Version 05/2009), cross-reactivity for the assay was as follows: oxycodone (100%); oxymorphone (30%); codeine (40%); hydrocodone (30%); hydromorphone (10%); morphine (7.6%); dihydrocodeine (5%); norcodeine (< 0.5%); noroxycodone (0.5%); and noroxymorphone (0.2%). The remaining 12,685 specimens (October 7–December 5, 2008) were tested with the DRI® Oxycodone Assay (Microgenics, Fremont, CA), a homogeneous enzyme immunoassay (EIA). The cutoff concentration was 50 ng/mL. Cross-reactivity for the assay was as follows: oxycodone (100%); oxymorphone (103%); noroxycodone (<0.1%); noroxymorphone (<0.1%); codeine (0%); norcodeine (0%); dihydrocodeine (0%); hydrocodone (0%); hydromorphone (0%); and morphine (0%).

Confirmation procedures

Prior to confirmation analysis, all specimens were hydrolyzed with β-glucuronidase according to a published procedure (1). Each specimen was treated with β-glucuronidase [Type L-II, *Patella vulgate* (keyhole limpet), Sigma Aldrich, St. Louis, MO] at pH 5.0 and incubated for 2 h at 60°C. Following hydrolysis, specimens were analyzed by a validated LC–MS–MS method for the 10 opiates and metabolites on an API 3200 tandem MS operating in positive electrospray mode (ESI) (Applied Biosystems/MDS SCIEX, Toronto, ON, Canada). The optimum conditions for analysis were as follows: curtain gas, 30 psi; collision-activated dissociation, 5 psi; heated nebulizer temperature, 600°C; gas 1, 75 psi; and gas 2, 65 psi. In order to establish the appropriate multiple reaction monitoring (MRM) conditions for individual compounds, solutions of standards in methanol/water (50:50, v/v) were infused into the MS, and the declustering potential (DP) and collision energy (CE) were optimized for the different ions. Data acquisition, peak integration, and calculation were interfaced to a computer workstation running Analyst 1.4.2 software (Applied Biosystems). Precursor and product ions (*m/z*) for the analytes and their respective internal standards were as follows: codeine, 300>152, 300>165; codeine-d_6, 306>115; norcodeine, 286>152, 286>165; (norcodeine was quantified with codeine-d_6); morphine, 286>152, 286>165; morphine-d_3, 289>165; hydrocodone, 300>199, 300>128; hydrocodone-d_6, 306>202; dihydrocodeine, 302>128, 302>199; dihydrocodeine-d_6, 308>202; norhydrocodone, 286>199, 286>241; (norhydrocodone was quantified with hydrocodone-d_6); hydromorphone, 286>185, 286>152; hydromorphone-d_6, 292>185; oxycodone, 316>298, 316>241; oxycodone-d_6, 322>247; noroxycodone, 302>284, 302>227; noroxycodone-d_3, 305>287; oxymorphone, 302>227, 302>198; and oxymorphone-d_3, 305>230.

Quality control samples were prepared in a urine matrix and analyzed with each batch of specimens. The four positive controls contained the following analytes: 1. 187.5 ng/mL of all analytes except morphine, 62 ng/mL morphine, 125 ng/mL morphine-3-glucuronide; 2. 375 ng/mL of all analytes except morphine, 125 ng/mL morphine, 250 ng/mL morphine-3-glucuronide; 3. 1250 ng/mL codeine, 375 ng/mL morphine, 937 ng/mL morphine-3-glucuronide; and 4. 2500 ng/mL codeine, 625 ng/mL morphine, and 1875 ng/mL morphine-3-glucuronide.

Criteria for identification and measurement were as follows: relative retention time difference was required to be within ±0.006 between relative retention time of analyte in a sample compared to the relative retention time of the same analyte in the calibrator; ion ratios for the product ions derived from analytes and internal standards in controls and donor specimens had to be within the ±20% mean range of those obtained from the corresponding substances in the calibrator; control samples had to measure within ±20% of the in-house determined mean value; and negative controls must not have analytes above the limit of quantitation (LOQ). The LOQ for all analytes was 50 ng/mL.

The upper limit of linearity (ULOL) for each analyte was determined by analyzing urine samples fortified with increasing drug concentrations. The ULOL was defined as the maximum concentration that could be accurately measured (±20% of target concentration) and also met all identification criteria. If specimens exceeded the determined upper LOQ, the specimens were diluted appropriately and reanalyzed. Drugs tested for potential interference included carisoprodol, meprobamate, propoxyphene, norpropoxyphene, tramadol, buprenorphine, norbuprenorphine, 7-aminoclonazepam, oxazepam, temazepam, lorazepam, hydroxyflurazepam, diazepam, alfentanil, sufentanil, fentanyl, norfentanyl, and methadone. No interferences from these or other drugs commonly employed in the management of pain were observed. Precision (%CV) and accuracy (%deviation) of control samples (free drug and hydrolysis controls) ranged from 3% to 7% and from –1% to 14%, respectively (25).

Results and Discussion

Prevalence of prescription opiates and related metabolites

Of the 20,089 specimens analyzed by LC–MS–MS, 6963 (34.7%) were negative, and 13,126 (65.3%) contained one or more of the 10 opiate analytes in the test panel at concentrations ≥50 ng/mL. The total number of analytes measured in the specimen set over the approximate 5-month period was 36,315. The number of opiate analytes detected per specimen ranged from 1 to 8, and the overall average was 4.0. Of the 13,126 positive specimens, the percentage of specimens containing one or more analytes were as follows (# analytes/%): 1/17.0; 2/23.7; 3/34.8; 4/17.1; 5/4.9; 6/1.8; 7/0.6; and 8/0.1.

Table I lists the prevalence and concentrations of the 10 opiate drugs and related metabolites in the 13,126 positive specimens. Hydrocodone was the most prevalent analyte followed in order by norhydrocodone, oxycodone, noroxycodone, oxymorphone, hydromorphone, dihydrocodeine, morphine, codeine, and norcodeine. Median concentration was highest for morphine followed by noroxycodone, oxycodone, codeine, oxymorphone, norhydrocodone, hydrocodone, norcodeine, hydromorphone, and dihydrocodeine. These patterns of drug prevalence and concentrations for the commercially available opiate drugs are similar in order and magnitude to an earlier study of drugs in chronic pain patients (1).

A total of 260 different drug/metabolite combinations were identified for the 13,126 positive specimens. Table II list the 25 most frequently recorded drug and metabolite combinations. Oxycodone and hydrocodone in combination with their respective metabolites were most prominent followed by morphine and morphine combinations.

Table I. Opiate Drug/Metabolite Prevalence, Percent Positivity, and Concentrations Measured in Urine Specimens by LC–MS–MS in 13,126 Pain Patient Specimens

Analyte	# Positive Specimens	% Positive*	Mean Concentration ± SEM (ng/mL)	Median Concentration (ng/mL)	Range (ng/mL)
Codeine	267	2.0	5331.9 ± 927.7	1551.0	59–160,600
Norcodeine	50	0.4	2163.7 ± 774.9	310.5	57–30,587
Morphine	2704	20.6	22,738.6 ± 1038.7	9174.0	52–1,122,000
Hydrocodone	5595	42.6	2091.2 ± 46.3	966.0	50–71,830
Dihydrocodeine	3698	28.2	400.1 ± 8.2	239.0	50–6204
Norhydrocodone	5372	40.9	2600.6 ± 59.3	1209.0	50–72,990
Hydromorphone	4282	32.6	695.5 ± 31.8	292.0	50–51,110
Oxycodone	5046	38.4	5433.1 ± 218.7	2026.5	50–548,900
Noroxycodone	4757	36.2	9082.9 ± 194.5	4159.0	50–189,600
Oxymorphone	4544	34.6	4098.6 ± 109.4	1444.5	50–172,641

* % Positive was calculated as 100 × # positive specimens/13,126.

Evaluation of opiate normetabolites as biomarkers of parent drug use

The usefulness of norcodeine, norhydrocodone, and noroxycodone as biomarkers of parent drug use was examined in this study with respect to their frequency of detection and excretion patterns. Norhydrocodone and noroxycodone were detected in 40.9% (n = 5372) and 36.2% (n = 4757) of all positive specimens, respectively, whereas norcodeine was detected in 0.4% (n = 50) (Table I). These results are similar to those reported in a pilot study of 2654 pain patients with the exception that the prevalence of norhydrocodone in this study (40.9%) is somewhat higher than observed in the pilot study (22.1%) (25). Mean and median concentrations of normetabolites of hydrocodone and oxycodone exceeded parent drug concentrations whereas mean and median norcodeine concentration were in the range of 20–41% of codeine. The lower prevalence and concentration of norcodeine relative to the other two normetabolites is likely due to differences in the activity of other metabolic pathways competing with CYP3A4 *N*-demethylation of codeine. Importantly, the codeine structure contains a 6-hydroxyl group, which is readily conjugated forming codeine-6-glucuronide (16). It is likely the activity of the glucuronidation pathway limits formation of norcodeine. Although oxycodone has a 14-hydroxyl group that potentially could be conjugated, excretion studies in healthy subjects indicate that conjugation of oxycodone is relatively low (26). The two major alternate metabolic pathways for hydrocodone and oxycodone are *O*-demethylation at C-3 and 6-keto-reduction at C-6. As noted, *O*-demethylation activity is dependent upon CYP2D6 activity, which is widely variable among the population. The 6-keto-reduction pathway for hydrocodone and oxycodone leads to the production of epimers of hydrocodol and oxycodol; however, urinary excretion of hydrocodol and oxycodol, as compared to parent drug, appear to be low (14,27).

Table II. Twenty-Five Most Frequently Recorded Opiate Drug and Metabolite Combinations in Urine Specimens Recorded for 13,126 Pain Patient Specimens

Single/Multiple Drugs/Metabolites	# Specimens	Relative Frequency
OC/NOC/OM*	2289	1
HC/DHC/NHC/HM	1362	2
HC/DHC/NHC	776	3
MOR	691	4
HC/NHC	690	5
OC/NOC	679	6
HC/NHC/HM	570	7
NHC	382	8
MOR/HM	333	9
OC/OM	326	10
HC/DHC/HM	312	11
MOR/OC/NOC/OM	298	12
NOC/OM	262	13
NOC	261	14
OM	231	15
HC	228	16
OC	203	17
HM	197	18
HC/DHC	186	19
MOR/HC/DHC/NHC/HM	157	20
HC/HM	144	21
MOR/HM/OC/NOC/OM	119	22
MOR/OC/NOC	104	23
DHC/NHC/HM	95	24
NHC/HM	87	25

* Abbreviations: OC, oxycodone; NOC, noroxycodone; OM, oxymorphone; HC, hydrocodone; DHC, dihydrocodeine; NHC, norhydrocodone; HM, hydromorphone; and MOR, morphine.

The usefulness of monitoring the three opiate normetabolites was assessed by determining the prevalence of specimens that contained normetabolites in the absence of the parent drug. Obviously, the greater the prevalence of a normetabolite in absence of parent drug, the better suited the normetabolite is as a biomarker of parent drug use. Three combinations of drug and normetabolite are possible: (I) parent drug without normetabolite; (II) parent drug and normetabolite are present; and (III) only normetabolite is detected (with/without other metabolites). As shown in Table III, codeine was frequently detected without norcodeine (81.8%), and only eight (2.9%) of the codeine specimens were positive for norcodeine in the absence of codeine. Of the eight specimens that were positive for norcodeine in the absence of codeine, two were in combination with morphine and six were norcodeine only. Thus, 2.2% (6/275) of the total positives for codeine and/or norcodeine were positive for norcodeine in the absence of codeine and morphine. In contrast to codeine, norhydrocodone and noroxycodone were frequently present in combination with the parent drug and were detected in the absence of the parent drug in 14.4% and 12.2% of hydrocodone and oxycodone specimens, respectively. For specimens that were positive for norhydrocodone in the absence of hydrocodone, 38.2% were in combination with dihydrocodeine and hydromorphone and 61.8% were norhydrocodone only. Thus, 8.9% (583/6538) of the total positives for hydrocodone and/or norhydrocodone were positive for norhydrocodone in the absence of hydrocodone, hydromorphone, and dihydrocodeine. For specimens that were positive for noroxycodone in the absence of oxycodone, approximately 50% were in combination with oxymorphone and 50% were noroxycodone only; 6.1% (350/5748) of the total positives for oxycodone and/or noroxycodone were positive for noroxycodone in the absence of oxycodone and oxymorphone. Clearly, monitoring for the three normetabolites provides additional evidence of parent drug use both in the presence and absence of the parent drug. However, norhydrocodone and noroxycodone appear to be more broadly prevalent and more useful as biomarkers of parent drug use.

An additional principle worthy of consideration when interpreting specimen tests involving codeine, hydrocodone, and oxycodone use is the relative timecourse of elimination of parent drug compared to normetabolite. Given that opiate normetabolites tend to exhibit longer half-lives than the parent drug (12,16), it follows that detection of parent drug in the absence of normetabolite would generally occur shortly after recent drug ingestion before normetabolite is formed and becomes detectable. Eventually, sufficient normetabolite may be produced and would be found in combination with parent drug in urine. At even later times after drug ingestion when the parent drug has been cleared, only normetabolite may be present, possibly as a result of its longer half-life of elimination and also from accumulation from multiple dosing.

Screening and confirmation for opiates

Simultaneous screening and confirmation analyses of the 20,089 urine specimens from chronic pain patients for opiates provided a unique opportunity to evaluate patterns of prescription drugs and associated metabolites across the entire set of specimens without limitations imposed by initial screening followed by confirmation of presumptive positive specimens. In this study, all specimens were screened by opiates ELISA at a cutoff concentration of 100 ng/mL. Cross-reactivity information from the manufacturer indicated that the assay should sensitively detect codeine, morphine, hydrocodone, dihydrocodeine, and hydromorphone whereas detection of oxycodone and oxymorphone would be expected to occur only at high concentrations. Very low cross-reactivity was indicated for the metabolites, norcodeine, and noroxycodone. Cross-reactivity for norhydrocodone was not reported.

Because of the low cross-reactivity of the opiates ELISA

Table III. Urine Specimen Combinations Containing Parent Drug and/or Normetabolite

Drug/Metabolite	# Total Positives (Drug and/or Normetabolite)	Drug Present Without Normetabolite, # (%)	Drug and Normetabolite Present, # (%)	Normetabolite Present Without Drug, # (%)
Codeine/norcodeine	275	225 (81.8)	42 (15.3)	8 (2.9)
Hydrocodone/norhydrocodone	6538	1166 (17.8)	4429 (67.7)	943 (14.4)
Oxycodone/noroxycodone	5748	991 (17.2)	4055 (70.5)	702 (12.2)

Table IV. Combinations of Tandem Opiate and Oxycodone Screening and LC–MS–MS Results for 13,126 Pain Patient Urine Specimens

Results	*N*	%
Opiate ELISA/Oxycodone ELISA/LC–MS–MS		
Positive/Positive/Positive	3715	50.2
Negative/Negative /Negative	2400	32.4
Negative/Negative/Positive	175	2.4
Positive/Negative/Negative	49	0.7
Negative/Positive/Negative	26	0.4
Positive/Positive/Negative	25	0.3
Negative/Positive/Positive	670	9.0
Positive/Negative/Positive	344	4.6
Total	**7404**	**100.0**
Opiate ELISA/Oxycodone EIA/LC–MS–MS		
Positive/Positive/Positive	2766	21.8
Negative/Negative/Negative	4277	33.7
Negative/Negative/Positive	210	1.7
Positive/Negative/Negative	101	0.8
Negative/Positive/Negative	78	0.6
Positive/Positive/Negative	7	0.1
Negative/Positive/Positive	1266	10.0
Positive/Negative/Positive	3980	31.4
Total	**12,685**	**100.0**

screen with oxycodone, a sensitive assay was needed for its detection. In the current study, the first 7404 specimens also were tested in tandem with the opiate ELISA by oxycodone ELISA at a cutoff concentration of 100 ng/mL. Cross-reactivity information from the manufacturer indicated that the oxycodone ELISA should sensitively detect oxycodone, but higher concentrations of oxymorphone and other opiates also should be detected. For the next 12,685 specimens, the oxycodone ELISA was replaced by oxycodone EIA, a homogeneous assay with a cutoff concentration of 50 ng/mL. Cross-reactivity information from the manufacturer indicated that the oxycodone EIA should sensitively detect both oxycodone and oxymorphone but should be relatively insensitive to other opiates and metabolites.

A comparison of the opiate and oxycodone screening results to LC–MS–MS results is shown in Table IV for the two periods of testing with different oxycodone screening assays. Of the eight combinations possible when comparing three assay results (opiate screen/oxycodone screen/LC–MS–MS), there was an 82.6% overall agreement (sum of true positives and true negatives) for the opiate ELISA/oxycodone ELISA/LC–MS–MS combination and 55.5% overall agreement for the opiate ELISA/oxycodone EIA/LC–MS–MS combination. The lower agreement for the opiate ELISA/oxycodone EIA/LC–MS–MS combination was due primarily to the lower rate of positive results (Pos/Pos/Pos) of 21.8% and a higher rate of negative oxycodone results (Pos/Neg/Pos) of 31.4%. Examination of these 3980 specimens revealed that most specimens contained various combinations of morphine, codeine, hydrocodone, and related metabolites, and only 19 (0.5%) specimens in this group contained oxycodone-related analytes (oxycodone, oxymorphone, noroxymorphone). Consequently, these results reflect the higher specificity of the oxycodone EIA for oxycodone-related compounds as compared to the oxycodone ELISA and are consistent with their reported cross-reactivities.

The prevalence of paired false-negative screening results (negative opiate screen/negative oxycodone screen/positive LC–MS–MS) was relatively low for the opiate ELISA in combination with both oxycodone assays. The opiate ELISA/oxycodone ELISA/LC–MS–MS group had 2.4% false-negative results, and the opiate ELISA/oxycodone EIA/LC–MS–MS group had 1.7% false-negative results. Examination of these two groups of specimens revealed a high prevalence of specimens containing only norhydrocodone or noroxycodone without other detectable opiate analytes. For the opiate ELISA/oxycodone ELISA/LC–MS–MS (n = 175) and the opiate ELISA/oxycodone EIA/LC–MS–MS (n = 210) groups, 54.3% and 50.5% of the specimens contained only norhydrocodone or noroxycodone.

Given the potential importance of opiate normetabolites in interpretation of test results, an examination was undertaken of screening results for specimens containing only norhydrocodone or noroxycodone. These specimens present a unique challenge for detection if screening and confirmation assays are performed in typical serial fashion rather than in parallel fashion as in this study. Information from the manufacturers of the opiate ELISA, oxycodone ELISA, and oxycodone EIA indicate low cross-reactivity with norcodeine and noroxycodone and offer no information on cross-reactivity with norhydrocodone. There were no specimens detected by LC–MS–MS containing only norcodeine. There were 382 specimens containing only norhydrocodone and 261 specimens containing only noroxycodone. It should be noted that specimens positive for only norhydrocodone or noroxycodone could have contained other opiate analytes at concentrations < LOQ. In terms of screening results for these specimens, 64.9% (n = 248) of the 382 norhydrocodone (only) specimens tested positive in the opiate ELISA whereas 13.4% (n = 35) of the 261 noroxycodone (only) specimens were positive. The low detection rate for noroxycodone in the Opiate ELISA is consistent with its reported cross-reactivity.

Of the 135 norhydrocodone specimens tested with the oxycodone ELISA, 45.2% (n = 61) were positive. The remaining norhydrocodone specimens (n = 247) were tested with the oxycodone EIA; only 2.4% (n = 6) were positive. Of the 136 noroxycodone specimens tested by the oxycodone ELISA, 44.1% (n = 60) were positive. The remaining 125 noroxycodone specimens were tested with the oxycodone EIA; 85.6% (n = 107) were positive. In summary, specimens containing only norhydrocodone were detected at the highest frequency by the opiate ELISA (64.9%) followed by the oxycodone ELISA (45.2%) but were poorly detected by the oxycodone EIA (2.4%). Specimens containing only noroxycodone were detected at highest frequency by the oxycodone EIA (85.6%) followed by the oxycodone ELISA assay (44.1%) and were poorly detected by the opiate ELISA (13.4%).

Conclusions

This study of 20,089 urine specimens from chronic pain patients provided a unique opportunity to evaluate the prevalence of prescription opiates and metabolites, assess the usefulness of including normetabolites in the test panel, and compare opiate and oxycodone screening results to LC–MS–MS results. All specimens were tested simultaneously with two tandem screening assays (opiates and oxycodone) and LC–MS–MS. Approximately two-thirds of the specimens were positive for one or more of the 10 opiate analytes. Hydrocodone and oxycodone (together with related metabolites) were most prevalent followed by morphine. Because of the complex metabolic patterns of the opiates, the usefulness of norcodeine, norhydrocodone, and noroxycodone as biomarkers of parent drug use was assessed. Norcodeine was only infrequently detected whereas the prevalence of norhydrocodone and noroxycodone was approximately equal to the prevalence of the parent drug. A substantial number of specimens were identified that contained norhydrocodone (n = 943) or noroxycodone (n = 702) but not the parent drug. Clearly, these results establish the value of inclusion of normetabolites in the test panel as biomarkers of parent drug use. Although these specimens may have contained additional metabolites, a significant number were positive for normetabolite only in the absence of parent drug and other metabolites: 8.9% of specimens with indicators of hydrocodone use were positive only for norhydrocodone (ap-

proximately 1 in 11) while 6.1% of specimens with indicators of oxycodone use were positive only for noroxycodone (approximately 1 in 16). Interpretation of parent drug use could not have been made for these patients without testing for these unique normetabolites.

Evaluations were performed of an opiate screening assay and two oxycodone screening assays (oxycodone ELISA and oxycodone EIA) compared to LC–MS–MS. Comparison of the opiate ELISA and oxycodone ELISA results to LC–MS–MS revealed high agreement (82.6%) whereas testing with opiate ELISA and oxycodone EIA produced moderate (55.5%) agreement with LC–MS–MS. Greater selectivity with the oxycodone EIA (less cross-reactivity with other opiate analytes) appeared to be the cause of the lower overall agreement with the opiate ELISA. However, use of the opiate ELISA in tandem testing with either oxycodone screening assays resulted in low false negative results compared to LC–MS–MS. For those specimens containing only norhydrocodone, detection rates were highest for the opiate ELISA (64.9%) followed by oxycodone ELISA (45.2%) and were poor with the oxycodone EIA (2.4%). Detection rates for noroxycodone (only) specimens were highest with oxycodone EIA (85.6%) followed by oxycodone ELISA (44.1%) and were poor with the opiate ELISA (13.4%).

References

1. E.J. Cone, Y.H. Caplan, D.L. Black, T. Robert, and F. Moser. Urine drug testing of chronic pain patients: licit and illicit drug patterns. *J. Anal. Toxicol.* **32:** 530–543 (2008).
2. S.V. Otton, M. Schadel, S.W. Cheung, H.L. Kaplan, and E.M. Sellers. CYP2D6 phenotype determines the metabolic conversion of hydrocodone to hydromorphone. *Clin. Pharmacol. Ther.* **54:** 463–472 (1993).
3. S.V. Otton, D. Wu, R.T. Joffe, S.W. Cheung, and E.M. Sellers. Inhibition by fluoxetine of cytochrome P450 2D6 activity. *Clin. Pharmacol. Ther.* **53:** 401–409 (1993).
4. A.A. Somogyi, D.T. Barratt, and J.K. Coller. Pharmacogenetics of opioids. *Clin. Pharmacol. Ther.* **81:** 429–444 (2007).
5. H.L. Kaplan, U.E. Busto, G.J. Baylon, S.W. Cheung, S.V. Otton, G. Somer, and E.M. Sellers. Inhibition of cytochrome P450 2D6 metabolism of hydrocodone to hydromorphone does not importantly affect abuse liability. *J. Pharmacol. Exp. Ther.* **281:** 103–108 (1997).
6. M.R. Hutchinson, A. Menelaou, D.J. Foster, J.K. Coller, and A.A. Somogyi. CYP2D6 and CYP3A4 involvement in the primary oxidative metabolism of hydrocodone by human liver microsomes. *Br. J. Clin. Pharmacol.* **57:** 287–297 (2004).
7. B. Lalovic, B. Phillips, L.L. Risler, W. Howald, and D.D. Shen. Quantitative contribution of CYP2D6 and CYP3A to oxycodone metabolism in human liver and intestinal microsomes. *Drug Metab. Dispos.* **32:** 447–454 (2004).
8. S.H. Weinstein and J.C. Gaylord. Determination of oxycodone in plasma and identification of a major metabolite. *J. Pharm. Sci.* **68:** 527–528 (1979).
9. K.P. Leow and M.T. Smith. The antinociceptive potencies of oxycodone, noroxycodone and morphine after intracerebroventricular administration to rats. *Life Sci.* **54:** 1229–1236 (1994).
10. C.M. Thompson, H. Wojno, E. Greiner, E.L. May, K.C. Rice, and D.E. Selley. Activation of G-proteins by morphine and codeine congeners: insights to the relevance of O- and N-demethylated metabolites at mu- and delta-opioid receptors. *J. Pharmacol. Exp. Ther.* **308:** 547–554 (2004).
11. S.F. Zhou, C.C. Xue, X.Q. Yu, C. Li, and G. Wang. Clinically important drug interactions potentially involving mechanism-based inhibition of cytochrome P450 3A4 and the role of therapeutic drug monitoring. *Ther. Drug Monit.* **29:** 687–710 (2007).
12. T.H. Nieminen, N.M. Hagelberg, T.I. Saari, A. Pertovaara, M. Neuvonen, K. Laine, P.J. Neuvonen, and K.T. Olkkola. Rifampin greatly reduces the plasma concentrations of intravenous and oral oxycodone. *Anesthesiology* **110:** 1371–1378 (2009).
13. H.K. Lee, L.D. Lewis, G.J. Tsongalis, M. McMullin, B.C. Schur, S.H. Wong, and K.T. Yeo. Negative urine opioid screening caused by rifampin-mediated induction of oxycodone hepatic metabolism. *Clin. Chim. Acta* **367:** 196–200 (2006).
14. E.J. Cone, W.D. Darwin, C.W. Gorodetzky, and T. Tan. Comparative metabolism of hydrocodone in man, rat, guinea pig, rabbit, and dog. *Drug Metab. Dispos.* **6:** 488–493 (1978).
15. B. Lalovic, E. Kharasch, C. Hoffer, L. Risler, L.Y. Liu-Chen, and D.D. Shen. Pharmacokinetics and pharmacodynamics of oral oxycodone in healthy human subjects: role of circulating active metabolites. *Clin. Pharmacol. Ther.* **79:** 461–479 (2006).
16. Y. Caraco, J. Sheller, and A.J. Wood. Pharmacogenetic determinants of codeine induction by rifampin: the impact on codeine's respiratory, psychomotor and miotic effects. *J. Pharmacol. Exp. Ther.* **281:** 330–336 (1997).
17. J.M. Oyler, E.J. Cone, R.E. Joseph, Jr., and M.A. Huestis. Identification of hydrocodone in human urine following controlled codeine administration. *J. Anal. Toxicol.* **24:** 530–535 (2000).
18. E.J. Cone, H.A. Heit, Y.H. Caplan, and D. Gourlay. Evidence of morphine metabolism to hydromorphone in pain patients chronically treated with morphine. *J. Anal. Toxicol.* **30:** 1–5 (2006).
19. E.J. Cone, Y.H. Caplan, F. Moser, T. Robert, and D. Black. Evidence that morphine is metabolized to hydromorphone but not to oxymorphone. *J. Anal. Toxicol.* **32:** 319–323 (2008).
20. E.J. Cone, P. Welch, B.D. Paul, and J.M. Mitchell. Forensic drug testing for opiates. III. Urinary excretion rates of morphine and codeine following codeine administration. *J. Anal. Toxicol.* **15:** 161–166 (1991).
21. E.J. Cone, W.D. Darwin, and C.W. Gorodetzky. Comparative metabolism of codeine in man, rat, dog, guinea-pig and rabbit: identification of four new metabolites. *J. Pharm. Pharmacol.* **31:** 314–317 (1979).
22. J.M. Mitchell, B.D. Paul, P. Welch, and E.J. Cone. Forensic drug testing for opiates. II. Metabolism and excretion rate of morphine in humans after morphine administration. *J. Anal. Toxicol.* **15:** 49–53 (1991).
23. M.L. Smith, R.O. Hughes, B. Levine, S. Dickerson, W.D. Darwin, and E.J. Cone. Forensic drug testing for opiates. VI. Urine testing for hydromorphone, hydrocodone, oxymorphone, and oxycodone with commercial opiate immunoassays and gas chromatography–mass spectrometry. *J. Anal. Toxicol.* **19:** 18–26 (1995).
24. M. Balikova, V. Maresova, and V. Habrdova. Evaluation of urinary dihydrocodeine excretion in human by gas chromatography–mass spectrometry. *J. Chromatogr. B Biomed. Sci. Appl.* **752:** 179–186 (2001).
25. E.J. Cone, A. Zichterman, D.L. Black, B. Cawthon, T. Robert, and Y.H. Caplan. Urine testing for norcodeine, norhydrocodone, and noroxycodone facilitates interpretation and reduces false negatives. *Forensic Sci. Int.,* in press.
26. R. Poyhia, T. Seppala, K.T. Olkkola, and E. Kalso. The pharmacokinetics and metabolism of oxycodone after intramuscular and oral administration to healthy subjects. *Br. J. Clin. Pharmacol.* **33:** 617–621 (1992).
27. A. Baldacci and W. Thormann. Analysis of oxycodol and noroxycodol stereoisomers in biological samples by capillary electrophoresis. *Electrophoresis* **26:** 1969–1977 (2005).

Manuscript received September 7, 2009;
revision received September 29, 2009.