Tentative Identification of Novel Oxycodone Metabolites in Human Urine

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Abstract

Oxycodone is a semisynthetic codeine derivative that has been used both as an analgesic and antitussive. In the mid 1990s, OxyContin was introduced as a slow-release formulation of oxycodone for use in patients with moderate to severe chronic pain from such ailments as arthritis, vertebral disc disease, and cancer. Doctors wrote 6.9 million prescriptions for OxyContin from May 2000 through May 2001. Thus, it is no surprise that hospitals and medical examiners' offices across the country have seen an increasing number of admissions and deaths resulting from oxycodone abuse and overdose. The laboratory identifies oxycodone as part of its routine abused and therapeutic drug-testing procedures. Routine gas chromatographic analysis of bile or urine in many of these cases revealed unidentified peaks in the region of oxycodone that appeared to be oxycodone metabolites. In humans, the only documented metabolites of oxycodone are oxymorphone and N-desmethyloxycodone (noroxycodone). This study attempts to characterize these compounds as "presumptive" metabolites based on circumstantial evidence from known metabolic pathways of oxycodone in other species, as well as of other opiates and narcotic analgesics.

Introduction

Oxycodone is a semisynthetic codeine derivative that has been used both as an analgesic and antitussive. It is an opioid agonist with a high oral/parenteral potency ratio relative to most other opioid analgesics. This is generally thought to be due to the presence of the methyl group on the aromatic hydroxyl that prevents its conjugation with glucuronic acid in the liver (1). Oxycodone has approximately 0.5 to 0.7 times the analgesic potency of morphine (2) and has adverse effects that limit the maximum tolerated dose. Because of these properties, it has been frequently employed clinically in formulations containing other analgesics such as aspirin (Percodan®) or acetaminophen (Percocet®).

In December 1995, Purdue Pharma introduced oxycodone in a slow-release formulation (OxyContin) for use in patients with moderate to severe chronic pain from such ailments as arthritis, vertebral disc disease, and cancer. This formulation, considered a "breakthrough drug" for Purdue Pharma, accounted for 83% of their revenue in 2000 and 2001. Doctors have written 6.9 million prescriptions for OxyContin from May 2000 through May 2001, producing $1.27 billion in sales. It is no surprise that hospitals and medical examiners' offices across the country have seen an increasing number of admissions and deaths resulting from OxyContin abuse and overdose. OxyContin is manufactured in 10-, 20-, 40-, 80-, and 160-mg tablets (Purdue Pharma has recently suspended sales of the 160-mg formulation in response to the escalating abuse of this drug) designed to be taken every 12 h. There is no recommended dosage for OxyContin; it is dosed based on the patient's response, tolerance, and type of conversion (e.g., from oral morphine, parenteral morphine, or other opioid analgesics). Non "time-release" oxycodone is dosed at 5 mg (total dose) every 6 h. Those who abuse OxyContin usually crush it and then either "snort" or inject it.

Nationwide, over the past year, the Drug Enforcement Administration (DEA) cites 146 cases in which OxyContin was verified as the direct cause of death or a contributing factor and 318 deaths in which OxyContin was "most likely involved". We examined the records for deaths at the Office of the Chief Medical Examiner of the State of Maryland (OCME) over a three-year period from 1998 through 2000, in which toxicological studies showed the presence of oxycodone. The laboratory identifies oxycodone as part of its routine abused and therapeutic drug-testing procedures. In keeping with the rest of the country, oxycodone-positive cases have increased from 27 in 1998 to 41 in 1999 and 69 in 2000. Of all oxycodone-positive cases for this period (n = 137), death was attributed directly to oxycodone in 41.6% (n = 57). Of these, 35.1% (n = 20) were due to oxycodone alone, and 64.9% (n = 37) were due to oxycodone in combination with alcohol and/or one or more other drugs.

Routine gas chromatographic (GC) analysis of bile or urine in many of these cases revealed unidentified peaks in the region of oxycodone that appeared to be oxycodone metabolites. In humans, the only documented metabolites of oxycodone are oxymorphone and N-desmethyloxycodone (noroxycodone) (3,4).
The primary purpose of this study was to attempt to characterize these compounds as presumptive metabolites based on circumstantial evidence from known metabolic pathways of oxycodone in other species, as well as of other opiates and narcotic analgesics.

**Methods**

**Cases**
Cases containing oxycodone were identified from cases investigated by the OCME.

**Materials**
All drug standards, including the oxycodone standard, were obtained from Alltech-Applied Science (State College, PA). Noroxycodone was purchased from Cerilliant Corp. (Round Rock, TX). Alpha and beta oxycodol were kindly donated by Purdue Pharma (Stamford, CT). All other reagents were purchased from J.T. Baker (Phillipsburg, NJ) or Aldrich Chemical Company, Inc. (Milwaukee, WI), and solvents were from Fisher Scientific (Fair Lawn, NJ) "Optima" grade.

**Extraction and instrumentation**
Oxycodone extraction was performed as follows: to 5 mL standard or urine specimen were added 2 mL 0.1N sodium hydroxide, 100 µL 100 mg/L mepivacaine/ethylmorphine (internal standard solution), and 2 mL n-butyl chloride. After mechanical rotation and centrifugation, the n-butyl chloride layer was separated and extracted with 3 mL 1N sulfuric acid. The acid layer was removed, alkalinized with 0.5 mL ammonium hydroxide, and extracted with 5 mL methylene chloride. The methylene chloride was transferred to a conical centrifuge tube, and 200 µL isopropanol was added. The methylene chloride was evaporated to the isopropanol layer at 40°C, which was then transferred to an autosampler vial for GC analysis.

Analysis was performed on a Hewlett-Packard (Palo Alto, CA) 5890 GC equipped with a nitrogen-phosphorus detector (NPD). The column was a J&W Scientific (Folsom, CA) DB-5MS 5% phenyl-methyl-silicone fused-capillary column (20 m x 0.18-mm i.d., 0.18-µm film thickness). Helium was the carrier gas flowing at 1 mL/min. The injector temperature was 260°C with a detector temperature of 280°C. The oven temperature began at 100°C, was held for 1.0 min, increased at 30°C/min to 200°C, then increased at 10°C/min to 260°C, and finally increased at 20°C/min to 300°C, which was held for 8 min. Splitless injection mode was used.

Drug confirmation was performed using a Hewlett-Packard 5890 GC equipped with a nitrogen-phosphorus detector (NPD). The column was a J&W Scientific (Folsom, CA) DB-5MS 5% phenyl-methyl-silicone fused-capillary column (20 m x 0.18-mm i.d., 0.18-µm film thickness). Helium was the carrier gas flowing at 1 mL/min. The injector

<table>
<thead>
<tr>
<th>Case</th>
<th>Oxycodone</th>
<th>Metabolites Detected*</th>
<th>Other Drugs</th>
<th>Cause/Manner of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>1, 2, 3, 4, 5, 6, 6</td>
<td>None</td>
<td>GSW/Suicide</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>2, 3, 6</td>
<td>Ethanol 0.06 mg/dL, Acetaminophen = 76</td>
<td>Drug Intoxication/ Undetermined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diazepam = 0.3, Hydrocodone = 0.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Not detected</td>
<td>1, 2, 3, 4, 6</td>
<td>Fluoxetine = 0.7, Norfluroxetine = 0.6</td>
<td>Chronic narcotism/Natural</td>
</tr>
<tr>
<td>4</td>
<td>2.9</td>
<td>1, 2, 3, 5</td>
<td>Ethanol = 0.14</td>
<td>Drug &amp; Alcohol Intox./ Undetermined</td>
</tr>
<tr>
<td>5</td>
<td>0.3</td>
<td>1, 3 (Bile)</td>
<td>Ethanol = 0.13, Acetaminophen = 52</td>
<td>Narcotic &amp; Alcohol Intox./Undetermined</td>
</tr>
<tr>
<td>6</td>
<td>0.23</td>
<td>1, 2, 5, 6</td>
<td>Ethanol = 0.1</td>
<td>Narcotic &amp; Alcohol Intox. Undetermined</td>
</tr>
<tr>
<td>7</td>
<td>Not detected</td>
<td>1, 2, 5, 6</td>
<td>Morphine (free) = 0.43</td>
<td>Narcotic Intoxication/ Undetermined</td>
</tr>
<tr>
<td>8</td>
<td>0.1</td>
<td>1, 5, 5</td>
<td>Morphine (free) = 0.42</td>
<td>Narcotic Intoxication/ Undetermined</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acetaminophen = 11, Meprobamate = 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diazepam = 0.3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.09</td>
<td>None detected</td>
<td>Morphine (free) = 1.1, Meperidine = 0.08</td>
<td>Narcotic Intoxication/ Accident</td>
</tr>
<tr>
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<td>Norfluroxetine = 0.07, Amitriptyline = 0.2</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.8</td>
<td>1, 2, 3, 4</td>
<td>Ethanol = 0.01</td>
<td>Drug Intoxication/ Undetermined</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>None detected (bile)</td>
<td>Ethanol = 0.05, Acetaminophen = 68</td>
<td>Drug Intoxication/Suicide</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imipramine = 4.2, Desipramine = 0.8</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fluoxetine = 3, Flurazepam = 43</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Norfluroxetine = 2.5</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.1</td>
<td>2</td>
<td>Morphine (free) = 0.44</td>
<td>Narcotic Intoxication/ Undetermined</td>
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<tr>
<td>13</td>
<td>3.9</td>
<td>1, 2, 3, 4</td>
<td>Doxylamine = 0.6</td>
<td>Drug Intoxication/ Undetermined</td>
</tr>
<tr>
<td>14</td>
<td>Not detected</td>
<td>1, 2, 3, 4</td>
<td>None</td>
<td>Injuries complicated by pulmonary embolism/Accident</td>
</tr>
</tbody>
</table>

* Oxycodone metabolites: 1, oxymorphone; 2, noroxycodone; 3, 14-OH-dihydrocodeine (α-oxycodol); 4, 14-OH-dihydrocodeine (noroxydol); 5, oxycodone-N-oxide; and 6, acetylated (presumptive).

* Presumptive.
5890 series 2 GC equipped with a 5972 mass selective detector. Similar chromatographic conditions to those listed were used. The mass spectrometer (MS) was operated in the scan electron impact mode.

Oxymorphone analysis was performed by a previously published procedure (5).

Results

Fourteen cases autopsied at the OCME were included in this study. Each case was tested for alcohol and therapeutic and abused drugs by a combination of color tests, immunoassay, and GC. Oxycodone was identified on the alkaline drug screen using GC-NPD and subsequently quantitated. The presence of oxycodone was confirmed by full-scan electron-ionization GC-MS.

Oxycodone concentrations in blood, as well as other drug concentrations and case information are listed in Table I.

Discussion

There have been limited pharmacokinetic studies of oxycodone in humans. Poyhia et al. (3) reported on the metabolism of oxycodone after intramuscular (IM) and oral administration. Oxycodone was administered to nine volunteers in a cross-over study, each receiving three doses of oxycodone at two-week intervals: 0.14 mg/kg IM once and 0.28 mg/kg orally twice. They found no significant difference in maximum plasma concentrations between the three treatments, which varied from 0.034 to 0.038 mg/L. The median t_{max} was 1 h in all groups. They found the bioavailability of oral relative to IM was 60%. They reported recovery of only oxycodone, oxymorphone, and noroxycodone in the urine.

Also in 1992, Leow et al. (2) reported on the comparative oxycodone pharmacokinetics in humans after intravenous (IV), oral, and rectal administration. They gave single doses of 4.6–7.3 mg IV, 9.1 mg (free base) syrup orally, two 5-mg tablets orally, or a suppository containing 30 mg oxycodone base rectally to 4 groups of 12 patients each. The IV doses gave peak plasma concentrations of 0.028–0.108 mg/L at 0.03–0.5 h postdosing. Maximum plasma concentrations following the 2 x 5-mg tablets and the 9.1 mg syrup were 0.013–0.046 mg/L and 0.014–0.033 mg/L, respectively. The mean t_{max} were 1.48 and 1.80 h, respectively. Peak plasma concentrations of 0.048–0.118 mg/L were reached with the rectal suppository at a mean t_{max} of 3.12 h.

In humans, the only documented metabolites of oxycodone are oxymorphone and N-desmethylxoycdone (noroxycodone) (3,4). Our data also indicate that oxymorphone and noroxycodone were the two most commonly detected metabolites. Oxymorphone was detected in 9 of 12 cases (cases 2 and 12 had insufficient sample for oxymorphone analysis); noroxycodone was detected in 10 of 14 cases.

Studies of other opioids have identified metabolites in addition to those that are structural analogues of oxymorphone and noroxycodone. For example, Cone et al. (6) reported the formation of hydrocodol and hydromorphol following hydrocodone use in humans. New metabolic pathways for morphine resulting in novel metabolites have recently been discovered in guinea pigs (7,8). Additionally, Yeh et al. (9) reported N-oxide formation from morphine and normorphine as a product of oxidation of a tertiary amine by amine oxidase. N-Oxides of narcotics have also been isolated in vitro (10). The amine oxidase is present in the liver of humans and catalyzes the oxidation of tertiary amines to N-oxides and secondary amines to hydroxamines.

Ishida et al. (1,7) published their discovery of nine urinary products (including unchanged oxycodone) in the urine of rabbits; eight of them were isolated and identified. The metabolic pathways included O-demethylation, N-demethylation, 6-keto-reduction, N-oxide formation, and 7β-hydroxy-6β-oxycodol formation. None of these metabolites have as yet been reported in humans.

Routine urine drug screening for alkaline drugs in several postmortem cases in which oxycodone was detected revealed, in addition to parent drug, the presence of several unidentified peaks in the vicinity of oxycodone with both NPD and MS. Figure 1 shows the total ion chromatogram from a urine extract of an oxycodone case. In Figure 1, the peak labeled “OXC” corresponds to oxycodone. The spectrum (Figure 2) has a base peak that is m/z 315 with other prominent ions at m/z 230, 201, and 258.

To tentatively identify the remaining peaks, the mass spectra were studied to ascertain a relationship with the mass fragmentation pattern of oxycodone and other opioid compounds.
(11). By combining this information with known routes of metabolism, a tentative identification of these compounds could be made.

Compound 2 (Figure 3) is the easiest to decipher. It has a base peak of $m/z$ 301 with other prominent ions at $m/z$ 216 and 188. This indicates the loss of a methyl group. Because oxycodone has an O-methyl and N-methyl group, it is clear that one of these methyl groups is lost. Therefore, this peak corresponds to oxymorphone or noroxycodone. Because an authentic oxymorphone standard failed to produce a peak at that retention time, we concluded this peak corresponds to noroxycodone. Analysis of an authentic noroxycodone standard verified this identification.

Compound 3 (Figure 4) has a base peak of $m/z$ 317 with other ions at $m/z$ 300 and 230. The addition of 2 mass units to the parent drug suggests reduction of the ketone group, forming an alcohol. Ishida et al. (12) identified 14-OH-dihydrocodeine (oxycodol) as an oxycodone metabolite in rabbits. Because the $m/z$ 230 fragment involves the entire loss of the ring containing that oxygen, the 230 ion is consistent with this proposal. Analysis of an authentic standard confirmed the identification of this compound as $\alpha$-oxycodol. Consistent with this scheme, we hypothesized that peak 4 (Figure 5) is noroxycodol, the reduction product of noroxycodone, because it contains a $m/z$ 303 and $m/z$ 216.

Peak 5 in Figure 1 is a shoulder on the noroxycodone peak. The unique feature in this mass spectrum is a base peak of $m/z$ 331, or 16 mass units greater than oxycodone (Figure 6). Ishida et al. (12) reported the formation of an $N$-oxide in rabbits. Although $N$-oxide formation has rarely been reported in opioid compounds, Kassahun et al. (13) describe the formation of the 4'-$N$-oxide metabolite of olanzapine. Olanzapine's cyclic tertiary amine is structurally similar to the "D" ring of oxycodone. More mass spectral information could not be obtained from peak 5 because of the contribution of the larger noroxycodone peak. Cases 4, 6, 7, and 8 contained what we believe to be oxycodone-N-oxide.

Figure 7 depicts another chromatogram from an oxycodone case. In addition to oxycodone, noroxycodone, and the reduction metabolites discussed, there are several later peaks that have mass spectra as provided. We believe these could be acetylated metabolites, based on prominent ions at 341, 355, 357, and 389. The rapidly expanding field of "molecular pharmaco-
Acetylation is a relatively common reaction of primary amine (NH₂) groups, specifically aromatic and aliphatic amines, the α-amino groups of amino acids, the amine of hydrazines, and sulfonamides in the formation of and metabolic activation to mutagenic and carcinogenic electrophiles (17).

Acetylation is a relatively common reaction of primary amine (NH₂) groups, specifically aromatic and aliphatic amines, the α-amino groups of amino acids, the amine of hydrazines, and sulfonamido amines (17). Compounds that lack an appropriate amine group can have one unmasked or introduced. Caffeine is an example of a drug with a cyclic methylamine such as oxycodone that has been shown to undergo metabolic ring opening and conversion to a primary amine that is subsequently acetylated. Additionally, several reports have described the importance of acetyl-coenzyme-A-dependent O-acetylation of hydroxylamines. Because Yeh et al. (9) have already reported on the conversion of N-oxides to hydroxylamines by amine oxidases and olanzapine is also documented to produce an N-oxide metabolite, it is not inconceivable that the presumptive N-oxide oxycodone metabolites we have tentatively identified could be further metabolized to the hydroxylamine and subsequently acetylated. Artificially created acetyl derivatives of this class of compounds have prominent ions similar to those we have seen at 341 (codeine-AC), 343/387 (nordihydrocodeine-2AC), and 357 (oxycodone-AC).

Hereditary differences in the acetylation of drugs in humans have been well characterized (17). This trait is currently referred to as “acetylation polymorphism” because it describes the differing capacity to metabolize numerous drugs and chemicals via this pathway. Individuals are generally categorized as “rapid” and “slow” acetylators of amine and hydrazine drugs. This so-called “acetylator status” can explain the differing pharmacological and toxicological profile of many drugs such as hydralazine, procainamide, isoniazid, and certain sulfonamides. Additionally, acetylator status has been implicated as a factor in individual susceptibility to bladder, colorectal, and breast cancers. The percentages of rapid acetylators range from 90% to 95% in Japanese and Eskimos, 78% of Chinese, and 72% of Thai origin to 20% or less of Egyptians and certain Jewish groups. Europeans and peoples of African origin have intermediate percentages of rapid acetylators. One study of tuberculosis patients living in the United States (18) found 55% were slow acetylators and the remaining 45% rapid acetylators. We detected what we believe to be acetylated metabolites in only 3 of the 14 cases reported here. None of these individuals were of Asian descent. This small percentage of cases in which these presumptive metabolites were detected may be due to the relatively small percentage of rapid acetylators among peoples of European or African descent or may be a factor of the stability of these metabolites in urine.

A metabolic pathway summarizing the production of the listed confirmed and proposed metabolites is presented in Figure 8 (13,19–22).

In recent years, it has become increasingly apparent that the pharmacoki-
hetics/pharmacodynamics of a compound cannot be completely understood until the products of that compound's metabolism have also been studied. Metabolites of some drugs/compounds have been found to add to the toxicity of the parent compound (e.g., methanol, glutethimide, acetaminophen, cocaine, and the ampheta-mine-like stimulants) or, in many cases, metabolites may increase the potency of the parent drug (e.g., many of the benzodiazepines and procainamide). Before these effects can be characterized, however, the metabolites must be identified. For these reasons, we believe the work reported here has inestimable value in beginning to understand the varied effects of compounds such as oxycodone and may have some benefit in the understanding of phenomena such as tolerance.

It should be noted that the metabolites of oxycodone discussed are those compounds that eluted from a DB-5 column after an alkaline liquid-liquid extraction with no derivatization. This discussion does not include any analogous metabolites of oxymorphone that would require derivatization for analysis. This study also did not include any phase 2 metabolic products.

We also emphasize that much of the discussion refers to a proposal of previously unrecognized metabolites for oxycodone. This proposal is based on both the known metabolic patterns of similar compounds and mass spectral analysis. Definitive identification of one previously unreported oxycodone metabolite in humans, alpha-oxycodol, has been completed. Conclusive identification of the other proposed metabolites will require their synthesis and subjecting these synthetic compounds to the same analytical scheme that was utilized for these case bile and urine specimens. It is hoped that future work will enable this to occur.

Figure 8. Proposed metabolic pathways for the production of the confirmed and novel oxycodone metabolites.

References


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