The Detection of Oxycodone in Meconium Specimens

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Abstract

A procedure for the determination of oxycodone in meconium using direct ELISA microplate technology followed by electron impact gas chromatography–mass spectrometry (GC–MS) is described for the first time. The abuse of oxycodone (OxyContin™) has been widely discussed in mainstream media, and it has been described as a cheap form of heroin. Oxycodone has been reported as having a high degree of abuse and potential complications in neonates from maternal drug use. Using a standard enzyme multiplied immunoassay screening technology, the cross-reactivity of oxycodone to the morphine antibody is only 5–6%. A positive screening value would require a high concentration of drug to be present, so a protocol for the detection of oxycodone in meconium using a direct ELISA microplate immunoassay followed by GC–MS was developed. The assay is now routinely used in our laboratory.

Introduction

Oxycodone (14-hydroxy-7,8-dihydrocodeinone) is a semi-synthetic opioid derived from the opium alkaloid thebaine. Marketed as OxyContin and Roxicodone™, oxycodone is a strong opioid agonist that is available alone or in combination with mild analgesics. It is suitable for oral and nasal administration because of its high bioavailability (50–65%), which makes it a good candidate for nasal abuse (1). It can also be given intramuscularly, intravenously, subcutaneously, and rectally. In analgesic potency, oxycodone is comparable to morphine, and with the exception of hallucinations, which may occur less frequently after oxycodone than after morphine, the side effects of these drugs are closely related. The abuse potential of oxycodone is equivalent to that of morphine.

In a study of the pharmacokinetics and metabolism of oxycodone in nine healthy young volunteers, the concentrations of oxycodone, noroxycodone, and oxymorphone in plasma and the 24 h urinary recoveries of their conjugated and unconjugated forms were measured. Plasma oxymorphone concentrations were below the limit of the assay. The study found no statistical differences in the various pharmacokinetic parameters for oxycodone between when intramuscular and oral administration were compared. Eight to 14% of the dose of oxycodone was excreted in the urine as unconjugated and conjugated oxycodone over 24 h. Oxymorphone was excreted mainly as a conjugate whereas noroxycodone was recovered mostly in an unconjugated form (2). Unfortunately, there are no pharmacokinetic data available for the metabolic profile of oxycodone in meconium.

As with all pure opioid agonists, there is no “ceiling effect” to the analgesia, so patients can continue to increase their dosages indefinitely as tolerance builds and maintain analgesic effect. Its most dangerous potential side effect is the respiratory depression by direct action on the brain stem respiratory centers. It depresses the cough reflex by action on the cough center in the medulla, produces nausea, causes miosis even in total darkness, and slows digestion of food in the small intestine. Headache, dry mouth, constipation, somnolence, sweating, and dizziness have also been reported by some users (3).

Many hospitals employ routine testing of neonatal and/or maternal specimens for the determination of drug and alcohol use during pregnancy. Although neonatal urine is widely tested, it gives only a short history of maternal drug use. Meconium, the first fecal material passed by a newborn, extends the window of drug detection up to 20 weeks and has become widely accepted as an alternative to urinalysis. In addition to morphine and codeine, there have been reports of heroin metabolites (4) and hydrocodone and hydromorphone (5) in meconium, but to date there are no reports of the metabolism, deposition, or detection of oxycodone or its metabolites in either meconium or neonatal urine. There is one report of its presence in breast milk (6). Because oxycodone has been increasingly identified as a potent narcotic resulting in drug dependence, overdose, and death, its use during pregnancy may result in withdrawal symptoms in the newborn (7). Hospitals are increasingly requesting the analysis of oxycodone in meconium specimens. Because the cross-reactivity of oxycodone with our standard screening technology (EMIT) is only 5–6% (8), specific microplate and gas chromatography–mass spectrometry (GC–MS) assays were modified for use with meconium specimens, for the routine analysis of oxycodone in specimens received into our laboratory.

Materials and Methods

Reagents

All reagents and chemicals were of analytical grade or better. Methoxyamine hydrochloride was purchased from Sigma.
were high-performance liquid chromatography grade or better. Acetylmorphine-\textsuperscript{d3}, hydrocodone-\textsuperscript{d3} and oxycodone d\textsuperscript{3}) as well as unlabeled drug standards were obtained from Cerilliant, (Austin, TX). All drugs were obtained in 1 mg/mL solutions in methanol, except 6-acetylmorphine and deuterated 6-acetyl-morphine, which were in acetonitrile. The final internal standard concentration was 2 \mu g/mL.

Sample preparation

Calibrators and controls. Calibrators and controls, for both screening and confirmatory procedures were prepared in a meconium matrix. The calibrator was spiked at a concentration of 100 ng/g; and the positive controls were spiked at 200 ng/g by the addition of a known amount of oxycodone to previously analyzed drug-free meconium specimens.

Specimens. Calibrators, controls, or meconium specimens (approximately 0.5 g) were weighed into polypropylene tubes and centrifuged for 5 min at 2000 rpm. Methanol (1 mL) was added, and the specimen was homogenized. The sample was mixed and centrifuged again for 5 min at 2000 rpm, the supernatant was poured into a separate tube, and 0.1M phosphate buffer (pH 6.0) was added (12 mL). Clean Screen DAU extraction columns were conditioned by sequentially passing methanol (3 mL), deionized water (3 mL), and 1.93M acetic acid (1 mL). The sample was added to the column, and the column was allowed to dry. The column was rinsed with deionized water (3 mL) and 0.1M HCl (1 mL). The acidic drugs were eluted. The column was then washed with methanol (3 mL) and dried for 5 min at full vacuum. The oxycodone was eluted in the basic fraction using fresh elution solvent (95% methylene chloride/isopropanol, 5% ammonium hydroxide) (3 mL). The eluent was evaporated to dryness, adding 1 drop of 0.2% succinic acid after the first 5 min and after the next 5 min. The specimen was reconstituted in 250 \mu L of buffer and transferred to the screening area.

Screening

The Immunalysis Oxycodone Direct ELISA is based upon competitive binding to antibody of enzyme labeled antigen and unlabeled antigen, in proportion to their concentration. An aliquot of the meconium extract (25 \mu L), calibrator or control was diluted with 200 \mu L of Immunalysis buffer which is supplied with the screening kit, and contains 100mM PBS with protein stabilizers and surfactants. Fifteen microliters was added to the microplate well, previously coated with a fixed amount of high affinity purified polyclonal antibody. The extracts were incubated for 1 h in the dark at room temperature with 100 \mu L of enzyme labeled oxycodone derivative (enzyme conjugate). The wells were washed six times with deionized water and inverted to dry. Substrate reagent (100 \mu L) was added, and the plate was incubated in the dark at room temperature for 20 min. Stopping solution was added (100 \mu L) to halt the reaction, and the absorbance value was read at a dual wavelength of 450 and 620 nm.

Confirmation

Presumptive positive samples identified using the results from the Oxycodone Direct ELISA screening kit were carried forward to confirmation using GC–MS.

Deuterated internal standard (100 \mu L) was added to an aliquot (1.0 g) of each calibrator, control, or meconium specimen. The internal standard concentration of deuterated oxycodone was 200 ng/g. The extracted calibrator concentration was 100 ng/g for all drugs, and the positive control was spiked at 200 ng/g. Methanol (3 mL) was added, the specimens were homogenized and centrifuged, and the supernatant was decanted into a small glass tube. The supernatant was evaporated to dryness at 60°C, and refrigerated overnight. The next day, 0.1M hydrochloric acid (3 mL) was added with 250 \mu L of 10% methoxyamine hydrochloride (aqueous). The mix was incubated at room temperature for 1 h and mixed; 0.1M phosphate buffer (pH 6.0, 3 mL) was then added.

Extraction procedure

An appropriate number of solid-phase mixed-mode extraction columns were placed into a vacuum extraction manifold. Each column was conditioned with methanol (3 mL), deionized water (3 mL), and 0.1M phosphate buffer (pH 6.0, 3 mL). The sample was allowed to flow through the column using no vacuum. When the sample had passed through the column, the bed was dried for 1 min at full vacuum. The column was washed with deionized water (3 mL), 0.1M hydrochloric acid (3 mL), and methanol (3 mL). The column was allowed to dry after each wash stage. Glass collection tubes were placed in the manifold, and the opiates were finally eluted with fresh methylene chloride/isopropanol/ammonium hydroxide (80:20:5, 3 mL). The extracts were evaporated to dryness under nitrogen at 17 psi at 60°C. Ethanol (100 \mu L) was added, and the specimens were mixed, transferred to autosampler vial inserts, and re-evaporated to dryness. The vials were capped, and the residue was reconstituted with isooctane (50 \mu L) and BSTFA + 1% TMCS (10 \mu L). The extracts were heated for 30 min at 80°C in dry heating block before GC–MS analysis.

Analytical procedure

The analytical procedure was a modified version of a previously described method (9). Briefly, an Agilent 5971 mass selective detector operating in electron impact mode was used for analysis. The GC column was a 5% phenyl-95% methyl silicone DB-5 MS (12-m length, 0.20-mm i.d., and 0.33-\mu m film thickness), and the injection temperature was
250°C. The injection mode was splitless, and the injection volume was 3 μL. The oven was programmed from 100°C for 1 min, ramped at 25°C/min to 230°C, and then it was ramped at 30°C/min to 250°C.

The transfer line was set at 270°C. The ions monitored were 419.4 and 420.4 for oxycodone-d3 and 416.4 and 417.4 for oxycodone. The other five opiates, hydrocodone, hydromorphone, 6-acetylmorphine, morphine, and codeine, which are also monitored in the GC–MS assay, did not interfere with the chromatography or separation of oxycodone.

Results and Discussion

There are no literature profiles regarding the metabolic profile of oxycodone in meconium, which is certainly a limiting factor to this assay. Only the parent drug, no oxycodone metabolites, is targeted using the confirmatory procedure.

Screening

The Oxycodone Direct ELISA immunoassay kit shows a 30–35% cross-reactivity to oxymorphone, codeine, and hydrocodone at a concentration of 500 ng/mL, in urine and blood, but little or no cross-reactivity with other opiates (10). Because, in this application, the oxycodone is extracted from the meconium with extensive sample pre-treatment before screening, and the meconium matrix itself is not introduced directly into the micro-plate well, it was not deemed necessary to do extensive cross-reactivity studies in a meconium matrix. The cross-reactivity rates should be similar to blood and/or urine.

Furthermore, because the confirmatory procedure monitors six opiates, a positive result caused by these alternative opiates would be identified through the GC–MS method.

The kit is intended for use with urine or blood, so its modification to meconium required optimization. By varying aliquot size and dilution volumes, a final optimization was achieved giving good separation at a cut-off concentration of 100 ng/g

Figure 1. Oxycodone micro-plate enzyme immunoassay performance for meconium specimens.

Figure 2. Chromatogram and SIM trace of a negative meconium specimen (A) and chromatogram and SIM trace of a meconium specimen containing 62 ng/g of oxycodone (B).
with a coefficient of variation of 5.4%. The results using an aliquot volume of 15 mL of a 1:9 diluted extract, at spiked concentrations of 0, 100, and 200 ng/g, are shown in Figure 1. Adequate separation from the negative control, producing a B/B₀ of 29% was achieved at 100 ng/g. Three standard deviations around the means from 0, 100, and 200 ng/g did not overlap.

Confirmation
The linear range of the GC–MS assay was from 0 to 1000 ng/g, and the coefficient of variation at 50 ng/g was 5.0%. The concentration of 50 ng/g was used as the limit of quantitation.

Since December 1, 2003, 74 meconium specimens received into the laboratory contained requests for oxycodone analysis. Three specimens screened positively for oxycodone (4.0%) and all three confirmed at concentrations of 62 ng/g, 224 ng/g, and 490 ng/g, showing the excellent specificity of the immunoassay. A typical chromatogram of a negative meconium specimen is shown in Figure 2A, and the chromatogram relating to the specimen closest to the LOQ of 50 ng/g is shown in Figure 2B (oxycodone present at 62 ng/g). This GC–MS assay allows for the detection of six opiates in one run because it was originally developed for use with hair and oral fluid samples (9). For increased specificity of the method, if requests for oxycodone in meconium continue to increase, the development of an assay for oxycodone and its metabolites, noroxycodone and oxymorphone, should be considered.

There are no published reports of oxycodone concentrations in meconium samples, so correlation with maternal use or abuse is not possible. Other opiates have been detected in meconium using immunoassays (11), HPLC (12), and GC–MS (4,5).

Although no specific reports of oxycodone on neonatal outcome have been published, there are many papers studying the effects of heroin, buprenorphine, methadone, or other opiates. It is estimated that 55–94% of infants born to opioid-dependent mothers in U.S. will show signs of withdrawal. Johnson et al. (13) recently reported that newborns exhibiting neonatal abstinence syndrome (NAS) have longer hospital stays, surprisingly showing that the duration of stay and requirement for treatment were greater in the infants exposed to methadone plus other drugs compared to those exposed to non-methadone opioids. Symptoms of NAS include tremors and irritability, sleep abnormalities, feeding problems, low birth weight, and seizures. It was also recently reported (14) that NAS associated with buprenorphine generally appears within 12–48 h, peaks at approximately 72–96 h, and lasts for 120–168 h. It has further been shown that methadone-maintenance treatment during pregnancy is associated with more consistent prenatal care, more normal fetal growth, and reduced fetal mortality. However, neonatal withdrawal from methadone appears to be more severe than from heroin, as judged by amount of medication required to control symptoms and duration of treatment.

The analytical procedure described details the determination of oxycodone in meconium specimens and may provide useful information to neonatologists and researchers studying the effects of opiates on newborns.

References

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