Case report

Negative urine opioid screening caused by rifampin-mediated induction of oxycodone hepatic metabolism

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Abstract

Introduction: Oxycodone has become widely used in the clinic for the treatment of chronic pain. This reflects its favorable pharmacokinetics and side effect profiles.

Case report: We report a 60-y-old man who had a clinically significant drug interaction between rifampin and oxycodone, resulting in 3 consecutive negative urine oxycodone screens in a 2-month period, suggesting non-adherence. A combination of urine opioid metabolite quantification by GC/MS and CYP genotyping confirmed that he was compliant with his oxycodone therapy. Determination of the complete oxycodone metabolite profile and the CYP3A4/5 and 2D6 genotype allowed the physician to be confident that the patient was compliant with the medication (and not diverting it) and to increase his oxycodone dose to optimize his pain control.

Conclusion: This case demonstrates how the combination of analytical toxicology and pharmacogenetic analyses enhances a physician’s ability to personalize drug therapy in patients with chronic pain syndromes.

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1. Introduction

Opioid analgesics are one of the most important therapeutic components in the treatment of moderate to severe pain according to the World Health Organization (WHO) pain ladder. The choice of potency of opioid and its dose should be optimized for individual patient analgesic requirements [1]. Oxycodone is a semi-synthetic opioid analgesic that has been used clinically since 1917 [2]. It has a lower incidence of nausea and hallucinations compared to morphine, thus oxycodone has become one of the most commonly prescribed opioids in the U.S. [3]. The bioavail-

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which is a minor metabolite, but has an analgesic potency approximately 10 times that of morphine after parenteral administration [10]. Oxycodone and oxymorphone undergo conjugation by uridine diphosphate glucuronosyl transferase enzymes (UGTs) to yield glucuronides, [11] which are renally excreted (Fig. 1). Oxycodone metabolism is highly dependent on CYP450 enzymes. Therefore, concurrent administration of inhibitors or inducers of hepatic drug metabolizing enzymes have the potential to cause clinically significant changes in oxycodone disposition [12–14].

Patients receiving chronic opioid analgesic therapy are often subjected to surveillance of compliance to ensure that they are not diverting or abusing the drug. The most commonly used drug surveillance process is to perform random urine screenings for the opioid that the patient is taking and also detect any additional intake of other drugs of abuse [15]. Both immunoassay and gas chromatography–mass spectrometry (GC/MS) techniques have been extensively used in the random screening of opioids in urine samples. However, the widely available and often routinely used immunoassays are subject to analytical interferences from commonly prescribed drugs (e.g., venlafaxine, fluoroquinolone antibiotics) [16]; in addition, immunoassays are generally less analytically sensitive than GC/MS [17]. In one case report, a patient was mistakenly suspected of diverting from his oxycodone treatment and nearly was refused opioid prescription when his urine immunoassay screen was negative for oxycodone. Repeated analysis using GC/MS found that the oxycodone concentration in the patient’s urine was appropriate [18].

In this report, we describe a patient who had 3 consecutive negative urine oxycodone screens determined by GC/MS while he was reportedly taking a combination of immediate and controlled-release oxycodone for chronic pain. We investigated a combination of potential contributory factors causing repeated negative urine oxycodone results, and it was determined that the patient was complying with his oxycodone therapy, but the drug was not detected in his urine by these routine analytical screens because of increased metabolism of the drug.

2. Case presentation

A 60- y-old male of Mediterranean origin with a prior left tibia/fibula fracture was referred by his plastic surgeon to the Dartmouth-Hitchcock Medical Center Pain Clinic for his pain management. He had undergone numerous reconstructive orthopedic and skin procedures as his surgery was complicated by infections and subsequent development of osteomyelitis. The patient complained of pain and numbness on the dorsum of his left foot. His past medical history revealed hypertension, hypercholesterolemia and chronic bronchitis. He had been consuming >6 beers/day before the injury but had reduced his ethanol consumption to 2 beers/...
day since he started the orthopedic reconstructive procedures. He was a smoker with a 2–3 packs per day history for 48 y. He gave up smoking when he was hospitalized for operative procedures on his left leg. The patient was taking the medications as listed (Table 1) at the time he visited the Pain Clinic in May 2005.

Over a period of 7 months from November 2004 to May 2005, 4 random urine samples were taken from the patient for opioid screening using GC/MS. The only positive urine opioid screen for oxycodone (195 µg/l, detection limit is 100 µg/l) was obtained in the first sample in November 2004. The 3 other subsequent urine opioid screens (2 in March and 1 in May 2005) were all negative for oxycodone. The patient was adamant that he was taking oxycodone (and this was corroborated by his spouse) as directed by his physician, but was not getting adequate pain relief. Collaborations between the provider, a clinical pharmacologist, the clinical laboratory, and further more detailed urine and pharmacogenetic analyses were performed to elucidate the cause of these findings.

3. Laboratory studies

3.1. Urine drug analysis studies

The fourth urine sample collected in May 2005 that was reported negative for oxycodone was additionally analyzed for oxycodone and its metabolites by National Medical Laboratories (Willow Grove, PA). Oxycodone and metabolites quantitations were performed using the SIM mode on an Agilent Technologies 5793 GC/MS (Palo Alto, CA). Total oxycodone/metabolites were tested following enzymatic hydrolysis with β-glucuronidase (*Patella vulgata*), while free oxycodone/metabolites were tested without this initial hydrolysis. Following the addition of deuterated internal standards, samples were treated with hydroxylamine to form the oxime derivatives and then extracted using a solid phase extraction procedure followed by formation of the TMS derivative (Table 2). This urine analysis showed no trace of oxycodone in the patient’s urine. Free oxymorphone was below the detection limit (100 µg/l) and the total oxymorphone concentration was found to be 190 µg/l. The free noroxycodone concentration in the urine was found to be 700 µg/l.

3.2. Pharmacogenetic studies

After obtaining verbal informed consent from the patient, genotyping for CYP2D6, CYP3A4 and CYP3A5 was performed. Genomic DNA was extracted from a venous blood sample obtained from this patient using the QIAamp DNA mini kit (Qiagen, Valencia, CA). CYP2D6 genotyping was performed at the Dartmouth Medical School using the Nanochip® Molecular Biology Workstation (MBW) (Nanogen, San Diego, CA). Two polymerase chain reactions (PCRs) were performed for the DNA sample. The first amplified a fragment from 2D6 and 2D8 to determine 2D6 duplication and deletion status. The second PCR amplified 3 different amplicons which were used to detect single nucleotide polymorphisms (SNPs). These amplicons were desalted and then electronically addressed to sites on a 100-site Nanochip® microarray using MBW Loader. Specific reporters for each allele were infused into the microarray in a sequential manner and detection of the addressed amplicons were performed using the Reader component of the MBW. CYP3A4/3A5 genotyping was performed at the Medical College of Wisconsin using the Pyrosequencing methodol-

Table 2  
GC/MS Analysis of oxycodone and metabolites in urine  

<table>
<thead>
<tr>
<th></th>
<th>Results (µg/l)</th>
<th>Detection limit (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxycodone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free</td>
<td>Not detected</td>
<td>100</td>
</tr>
<tr>
<td>Totalb</td>
<td>Not detected</td>
<td>50</td>
</tr>
<tr>
<td>Oxyomorphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free</td>
<td>Not detected</td>
<td>100</td>
</tr>
<tr>
<td>Totalb</td>
<td>190</td>
<td>50</td>
</tr>
<tr>
<td>Noroxycodone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free</td>
<td>700</td>
<td>50</td>
</tr>
</tbody>
</table>

b Total oxycodone or oxymorphone was analyzed following enzymatic hydrolysis with β-glucuronidase (*Patella vulgata*). Both conjugated and unconjugated forms were detected.

Table 3  
CYP2D6, CYP3A4 and CYP3A5 genotyping results  

<table>
<thead>
<tr>
<th>CYP2D6 gene duplication/deletion</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6*2</td>
<td>WT</td>
</tr>
<tr>
<td>CYP2D6*3</td>
<td>WT</td>
</tr>
<tr>
<td>CYP2D6*4</td>
<td>HT</td>
</tr>
<tr>
<td>CYP2D6*6</td>
<td>WT</td>
</tr>
<tr>
<td>CYP3A4*1B</td>
<td>WT</td>
</tr>
<tr>
<td>CYP3A5*3</td>
<td>HM</td>
</tr>
</tbody>
</table>

WT — wild type; HT — heterozygous mutant; HM — homozygous mutant.

Fig. 2. A sample of the patient’s urine (left) and the urine of an anonymous healthy volunteer (right).
ogy (Biotage, Uppsala, Sweden). PCR was performed on the sample and the Pyrosequencing analyses of CYP3A4 and CYP3A5 were then performed using a PSQ™HS96A system and software. Genotyping results are shown in Table 3. The patient was heterozygous for CYP2D6 *4, resulting in a CYP2D6 genotype of *4/*1, which was consistent with a phenotype of an intermediate metabolizer. The patient also had a functional CYP3A4 and non-functional CYP3A5 (homozygous variant), which yielded a phenotype of a normal metabolizer for CYP3A substrates.

3.3. Macroscopic urine appearance

The urine of the patient is shown in Fig. 2. Note the pinkish-orange color of the patient’s urine, which is consistent with rifampin ingestion, compared to the yellow-straw color of a normal subject not taking rifampin.

4. Discussion

We investigated the possible causes leading to 3 consecutive negative random urine screens for oxycodone and individualizing the patient’s drug therapy for optimal pain control. The patient was carefully interviewed to check for reported compliance. The patient and his spouse insisted that oxycodone was being taken according to the prescribing physician’s instructions. The presence of oxycodone metabolites in the urine proved that the patient was compliant with his oxycodone therapy. The high urinary noroxycodone and oxymorphone concentrations in this patient’s urine were expected as CYP3A4 was present in much larger quantities in the liver/gastrointestinal tract than is CYP2D6 [19,20]. Conjugated oxymorphone was found in excess of the free oxymorphone (unconjugated) as the majority of oxymorphone undergoes glucuronidation before being excreted in the urine [21]. Furthermore, the macroscopic observation that the patient’s urine had a pinkish-orange color was strongly suggestive that the patient was compliant with his rifampin therapy (Fig. 2). The most likely explanation therefore of this patient’s negative urine screens for oxycodone is rifampin-mediated induction of oxycodone metabolism. Thus, by having this patient’s urine screened for oxycodone at a reference laboratory that only detected parent oxycodone and not its metabolites, resulted in a series of false negative urine screening results.

Rifampin is a potent inducer of a number of isoenzymes in the CYP450 superfamily [22]. In vitro, rifampin causes a 55.1-fold induction of CYP3A4 activity, a 5-fold induction of CYP3A5 activity and 1.3-fold induction of CYP2D6 activity [23]. The onset of rifampin hepatic CYP enzyme induction is 1 to 3 days, while maximum induction of drug metabolism usually occurs after 9 to 12 days of chronic rifampin therapy [24]. Our patient had a single positive urine oxycodone screen 4 months before his 3 consecutive negative urine screens. A focused and detailed review of the patient’s medication record showed that he started taking rifampin for his surgical-wound infection a month before the first urine screen. At the time of the screen, he was also taking a higher dose of oxycodone (60 mg/day of controlled release oxycodone instead of 40 mg/day). The induction of oxycodone metabolism by rifampin should have reached its peak at the time of this positive screen, but a small amount of oxycodone was still detected in his urine as he was taking a larger dose of oxycodone. As the dose of oxycodone was subsequently reduced, the urine parent oxycodone screens turned negative because of its increased conversion to metabolites due to enzyme induction by rifampin.

To define whether pharmacogenomics was playing a role in the false negative oxycodone urine screen, a pharmacogenetics workup on this patient was conducted. It revealed that the patient was a CYP2D6 intermediate metabolizer with a 2D6 genotype of *4/*1. Therefore and most importantly, his CYP2D6 genotype was not that of an ultra-rapid metabolizer. Previous studies have suggested that rifampin induces CY2D6 only in extensive metabolizers and not in poor metabolizers [25,26]. Our patient is an intermediate metabolizer, so rifampin-induced metabolism of oxycodone can take place, but probably to a lesser extent than compared to an extensive metabolizer.

CYP3A4 and CYP3A5 genotyping revealed that the patient was wild type for CYP3A4 *1B and homozygous variant for CYP3A5 *3. This translates into a phenotype that this patient has non-functional CYP3A5 enzymes. CYP3A5 is only present in 20–30% of Caucasians [27] so it was not unexpected for this patient to be deficient in CYP3A5. Theoretically, it would be predicted that a person with non-functional CYP3A5 might metabolize certain drugs at a slower rate than patients with functional CYP3A5. However, there is a major overlap between CYP3A4 and CYP3A5 drug-substrate specificity, and CYP3A4 is more inducible than CYP3A5 [23]. Therefore, functionally there is usually more than adequate CYP3A4 capacity to catalyze drug metabolism in man. Interestingly, one report suggested that the oral clearance of midazolam was higher in healthy volunteers who were wild type for CYP3A4 *1B and homozygous variant for CYP3A5 *3 [28].

Our patient required rifampin for his osteomyelitis and oxycodone for his pain control. This case study demonstrated that rifampin induced CYP3A4 and CYP2D6 in this patient, causing an increased metabolism of oxycodone to its metabolites, in contrary to the common belief that CYP2D6 is either not inducible [29,30] or only slightly inducible [23]. The patient’s general compliance with his drug therapy regimen was confirmed by his pinkish-orange urine (due to rifampin ingestion) and positive oxycodone metabolite screens. Pharmacogenetics analyses revealed that the patient was an intermediate metabolizer for CYP2D6, wild type for CYP3A4 *1B and deficient in CYP3A5.

In summary, the combination of opioid metabolite detection and profiling, as well as CYP genotyping assisted in the confirmation that the patient’s negative
urine oxycodone screens were entirely due to rifampin-mediated induction of drug metabolizing enzymes. Since there are various urine oxycodone screens available commercially (some only detect parent compound, others detect parent and some metabolites), it is important that an institutional clinical laboratory is aware of the analytical sensitivities and specificities and limitations of their in-house or outsourced assays. The combination of drug metabolite studies and clinical pharmacology expertise permitted a data-driven recommendation to be made to the physician, who then confidently increased the patient’s dose of oxycodone to optimize his pain control while being continued on rifampin treatment. This case clearly illustrates the potential benefit of combining analytical detection of opioid metabolites with genotyping to personalize a patient’s drug therapy in the face of apparently inconsistent urine oxycodone drug screen data.

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References