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Article

Urinary Pharmacokinetics of Immediate and Controlled Release Oxycodone and its Phase I and II Metabolites Using LC–MS-MS

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Abstract

Oxycodone (OC) is a schedule II semisynthetic opioid in the USA that is prescribed for its analgesic effects and has a high potential for abuse. Prescriptions for OC vary based on the dosage and formulation, immediate release (IR) and controlled release (CR). Monitoring OC metabolites is beneficial for forensic casework. The limited studies that involve pharmacokinetics of the urinary excretion of OC metabolites leave a knowledge gap regarding the excretion of conjugated and minor metabolites, pharmacokinetic differences by formulation, and the impact of CYP2D6 activity on the metabolism and excretion of OC. The objectives of this study were to compare urinary excretion of phase I and II metabolites by formulation and investigate if ratio changes over time could be used to predict the time of intake. Subjects ($n = 7$) received a single 10 mg IR tablet of Oxycodone Actavis. A few weeks later the same subjects received a single 10 mg CR tablet of Oxycodone Actavis. During each setting, urine was collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 10, 12, 14, 24, 48 and 72 h. Urine samples (100 µL) were diluted with 900 µL internal standard mixture and analyzed on an Acquity UPLC® I-class coupled to a Waters Xevo TQD using a previously validated method. The CYP2D6 phenotypes were categorized as poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizers (UM). Comparisons between IR and CR were performed using two-tailed paired *t*-test at a significance level of $P = 0.05$. The metabolite ratios showed a general increase over time. Four metabolite to parent ratios were used to predict the time of intake showing that predictions were best at the early time points.

Introduction

In 2020, oxycodone (OC) was ranked seventh in the top 25 most identified drugs by state and local laboratories in the USA according to the National Forensic Laboratory Information System (NFLIS) 2020 midyear report (1). OC is a schedule II semisynthetic opioid that is commonly prescribed for pain relief despite its high potential for abuse. Prescriptions for OC can vary by dose and its formulation, immediate release (IR) and controlled release (CR). In 2017, opioids were the main cause of death in fatal poisonings in drug addicts in five Nordic countries. Of the drugs monitored, OC was the second most common intoxicant reported in Finland (2).

Monitoring metabolites of OC is beneficial for forensic casework. OC is primarily metabolized by CYP enzymes and further conjugated by uridine diphosphate glucuronosyltransferases (UGT). The main metabolite, noroxycodone (NOC), is produced via N-demethylation by CYP3A4 (3). OC is also metabolized by CYP2D6 to oxymorphone (OM) and by ketoreductase to the minor metabolites 6α-oxycodol (αOCL) and 6β-oxycodol (βOCL). Both NOC and OM are further metabolized to form noroxymorphone (NOM). OM and NOM undergo conjugation by UGT to form oxymorphone-3β-D-glucuronide (OMG) and noroxymorphone-3β-D-glucuronide (NOMG). Recently, a study by Jakobsson et al. investigated the CYP2D6 phenotypes in postmortem cases involving OC (4). The CYP2D6 phenotypes were categorized into four groups: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizers (UM). It was determined that PM and IM had significantly higher concentrations of OC and NOC in blood compared to EM and UM. Additionally, it was concluded that OM/OC ratios depended on CYP2D6 activity.

There are few studies on the urinary excretion of OC and its metabolites. The pharmacokinetics of OC, NOC and OM were investigated after subjects ($n = 9$) were administered

Table I. Demographics of Subjects

Subject	Gender	Age (years)	Weight (kg)	Height (cm)	Genotype	Phenotype
A	M	37	74	169	*1/*5	IM
B	F	22	58	171	*1/*4	IM
C	F	24	65	167	*1/*3	IM
D	F	22	73	173	*1/*4	IM
E	F	22	68	173	*4/*4	PM
F	F	25	83	177	*1/*1	EM
G	M	24	94	191	*3/*4	PM

0.14 mg/kg intramuscular OC and 0.28 mg/kg oral OC chloride in Poyhia et al. (5). Urinary NOC concentrations were significantly more in free form ($P < 0.01$) versus conjugated and OM concentrations were significantly higher as conjugated form ($P < 0.01$) rather than free.

In a study by Cone et al. subjects ($n = 12$) received a single 20 mg CR OC tablet and urine specimens were collected up to 52 h (6). The maximum concentration (C_{max}) values for the free OC, OM, NOC and NOM were 2,805, 149, 4,378 and 607 ng/mL, respectively. The OM C_{max} following hydrolysis was higher due to OM excretion as conjugate. The time to reach the maximum concentration (T_{max}) was 5–7.9 h for the free analytes. However, this study did not explore other formulations of OC and did not include other minor OC metabolites.

In Lalovic et al., the excretion of OC metabolites was monitored in subjects ($n = 16$) that were administered 15 mg OC over a 48-h period (7). The total urinary recovery of free OC, NOC, OM and NOM were 8%, 23.1%, 0.33% and 5.6% of total dose. The reduced metabolites, αOCL and βOCL, and their conjugates were excreted at 8% of the dose of OC.

The minimal studies that involve pharmacokinetics of the urinary excretion of OC metabolites leave a knowledge gap regarding the excretion of conjugated and minor metabolites, pharmacokinetic differences by formulation and the impact of CYP2D6 activity on the metabolism and excretion of OC. The overall aims of this study were to compare urinary excretion of phase I and II metabolites by formulation and investigate if ratio changes over time could be used to predict the time of intake.

Materials and Methods

Chemicals and reagents

NOMG, OMG, oxymorphone-3β-D-glucuronide-d_3 (OMG-d_3), NOM, OM, oxymorphone-d_3 (OM-d_3), NOC, noroxycodone-d_3 (NOC-d_3), OC and oxycodone-d_3 (OC-d_3) were obtained from Cerilliant (Round Rock, TX, USA), and αOCL and βOCL were purchased from from Cayman Chemical (Ann Arbor, MI, USA). Formic acid (98–100%) and acetonitrile (gradient grade) were acquired from Merck (Darmstadt, Germany). MilliQ® system (Millipore, Billerica, MA, USA) supplied purified water.

Human subjects

Urine samples were obtained from healthy volunteers ($n = 7$) as a part of a human pharmacokinetic study under a protocol approved by the Swedish Ethical Review Authority Dnr:2020–00102. Inclusion criterion was an age above 20 at the time of recruitment. Exclusion criteria were pregnancy, lactation, ongoing medication or involvement in another study at the same time. Demographics of the subjects are shown in Table I. Prior to enrollment, all participants gave written informed consent, provided a health declaration and underwent a medical examination.

Sample collection

The study was performed in two settings: first, subjects received a single 10 mg IR tablet of Oxycodone Actavis then a few weeks later the same subjects received a single 10 mg CR tablet of Oxycodone Actavis. During each setting, urine was collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 10, 12, 14, 24, 48 and 72 h. There was an exception for subject G, who only produced samples at 3, 4, 6, 10, 14, 24, 48 and 72 h for IR and 3, 4, 6, 10, 12, 24, 48 and 72 h for CR. Samples were refrigerated and analyzed within 72 h after the specimens were collected.

Urine analysis

Analysis was performed using a previously validated method (8). Urine (100 µL) was diluted with 900 µL internal standard mixture (prepared in MilliQ water) in an autosampler vial. Samples were analyzed on a Waters ACQUITY UPLC® I-class (Waters Co., Milford, MA, USA) equipped with Waters Xevo TQD. The limit of quantification (LOQ) was 0.015 µg/mL for OC, NOC, α/βOCL, OM and NOM and 0.050 µg/mL for NOMG and OMG. An AU680 (Beckman Coulter, Indianapolis, IN, USA) was used to determine creatinine concentrations as well as screening for OC using an enzyme immunoassay (DRI, Thermo Fisher Scientific, Hemel Hempstead, UK) with a threshold of 0.1 µg/mL urine. The cross reactivity for OC and OM was 100% and 103% whereas NOC and NOM showed less than 0.1% cross reactivity as stated by the manufacturer. The creatinine concentration was used to correct for differences in urine dilution.

Genotyping

The genotyping of CYP2D6 was performed by the Department of Clinical Chemistry and Pharmacogenetics at Uppsala University Hospital (Uppsala, Sweden). In brief, DNA was extracted from whole blood by QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) and amplified by ABI7500 Real-time Polymerase Chain Reaction (PCR) system (Applied Biosystems, Foster City, CA, US) with Taqman GTXpress Mastermix (Fischer Scientific, Waltham, MA, US). The investigated alleles were: *3, *4, *6 and *41. Copy Number Variation (CNV) analysis was performed to detect gene duplication, *2xn, and deletion of *5. The phenotypes PM, IM, EM and UM were predicted from the following combination of genotypes: PM *3/*3, *4/*4, *6/*6, *41/*41, *3/*4,

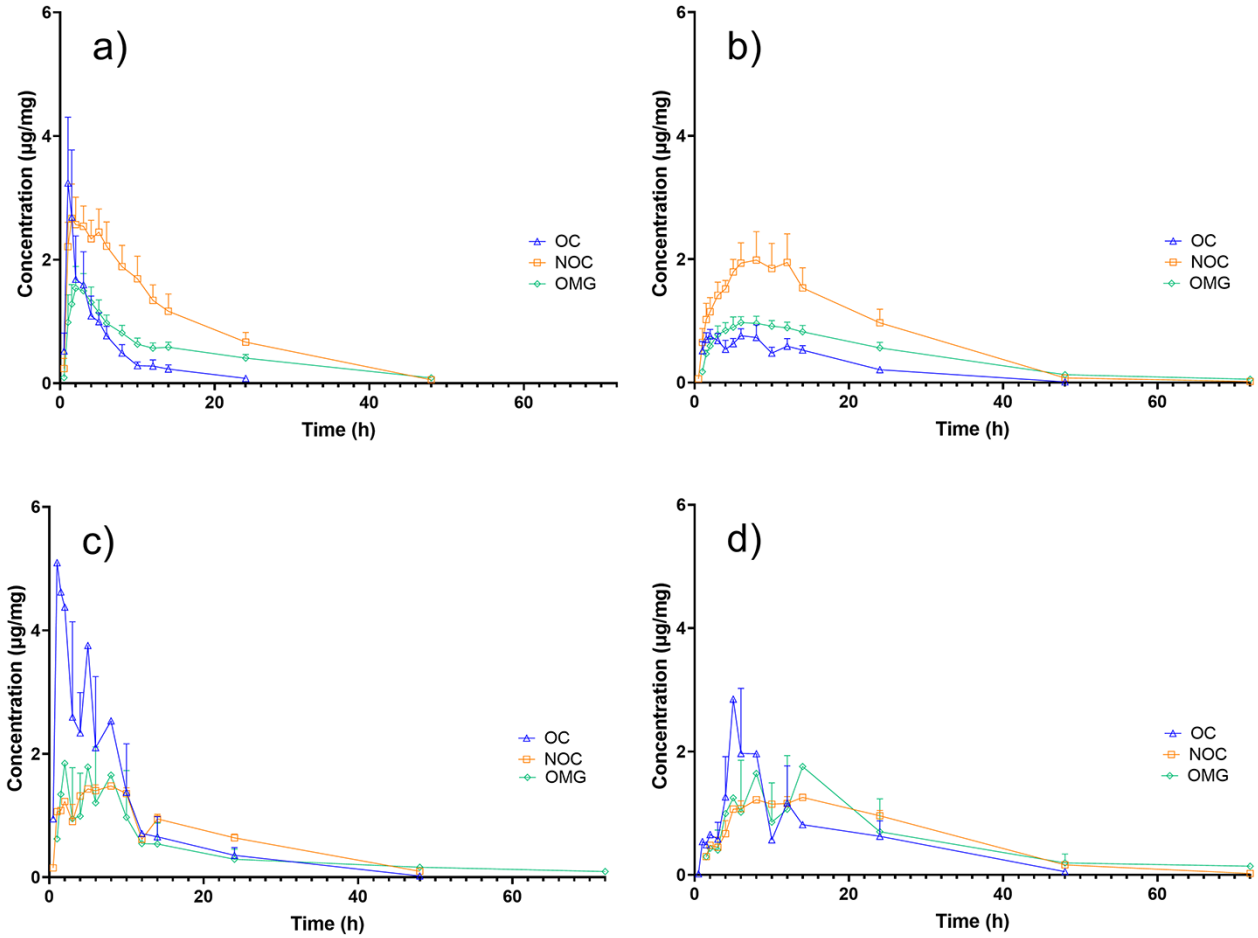

Figure 1. Time–concentration profiles normalized to creatinine after a single dose of 10 mg OC IR and CR of the major analytes (OC, NOC and OMG) grouped by phenotype: (a) IM/EM IR, (b) IM/EM CR, (c) PM IR, (d) PM CR. Data are presented as mean ± standard error of the mean (SEM).

*3/*6, *3/*41, *4/*6, *4/*41, *6/*41 or two nonfunctioning alleles, IM *1/*3, *1/*4, *1*5, *1/*6, 1/*41 or one nonfunctional allele and one *1, EM *1/*1, UM three or more functioning alleles.

Data analysis

Graph Pad Prism V.9 (San Diego, CA, USA) was used for statistical analyses. APL Pharmacokinetic Modeling Program (PKMP) was used for pharmacokinetic data analysis to determine non-compartmental parameters. Comparisons between IR and CR were performed using two-tailed paired *t*-test at a significance level of $P = 0.05$.

Application

To evaluate using metabolite ratios to estimate time of intake, an additional subject (subject H) was recruited (female, 23 years old, *1/*41 intermediate metabolizer). Urine was collected at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 10, 12, 14 and 24 h after administration of 10 mg of OC IR tablet. Additionally, urine from an authentic case was analyzed. A female driver (45 years old, unknown phenotype for CYP2D6) was suspected of driving under the influence of drugs with a blood sample positive for OC at 0.06 µg/g whole blood. Her prescriptions were OC CR, 5 mg, two tablets per day and OC IR 5 mg, max 3 tablets per day. She claimed to have taken three tablets of the IR 5 mg close to 3 h prior to the blood and urine sampling. The urine sample was analyzed and contained 26.2 µg/mL OC, 17.0 µg/mL NOC, 12.4 µg/mL OMG, 5.5 µg/mL βOCL, 1.7 µg/mL αOCL, 0.3 µg/mL NOM, 0.3 µg/mL OM and 0.13 µg/mL NOMG.

Results

Concentrations for OC and its metabolites are shown in Supplementary Table S1. Analyte concentrations from the LC–MS-MS analysis of IR and CR formulation are displayed as well as creatinine concentrations and OC values from immunoassay analysis. The detection of OC by the immunoassay was similar to the results from the LC–MS-MS method. The mean detection time for the immunoassay results was up to 41 h for IR formulation and up to 51 h for CR formulation. The 12-h sample was not collected from Subject A after CR dose. As previously mentioned, Subject G was unable to produce samples at 0, 0.5, 1, 1.5, 2, 5, 8 and 12 h for IR formulation and at 0, 0.5, 1, 1.5, 2, 5, 8 and 14 h for CR formulation.

Time-concentration profiles for the major analytes (OC, NOC and OMG) and minor analytes (αOCL, βOCL, NOM and NOMG) are displayed in Figures 1 and 2, respectively. The profiles are grouped by formulation type (IR and CR) and by phenotype groups (IM/EM and PM). As displayed

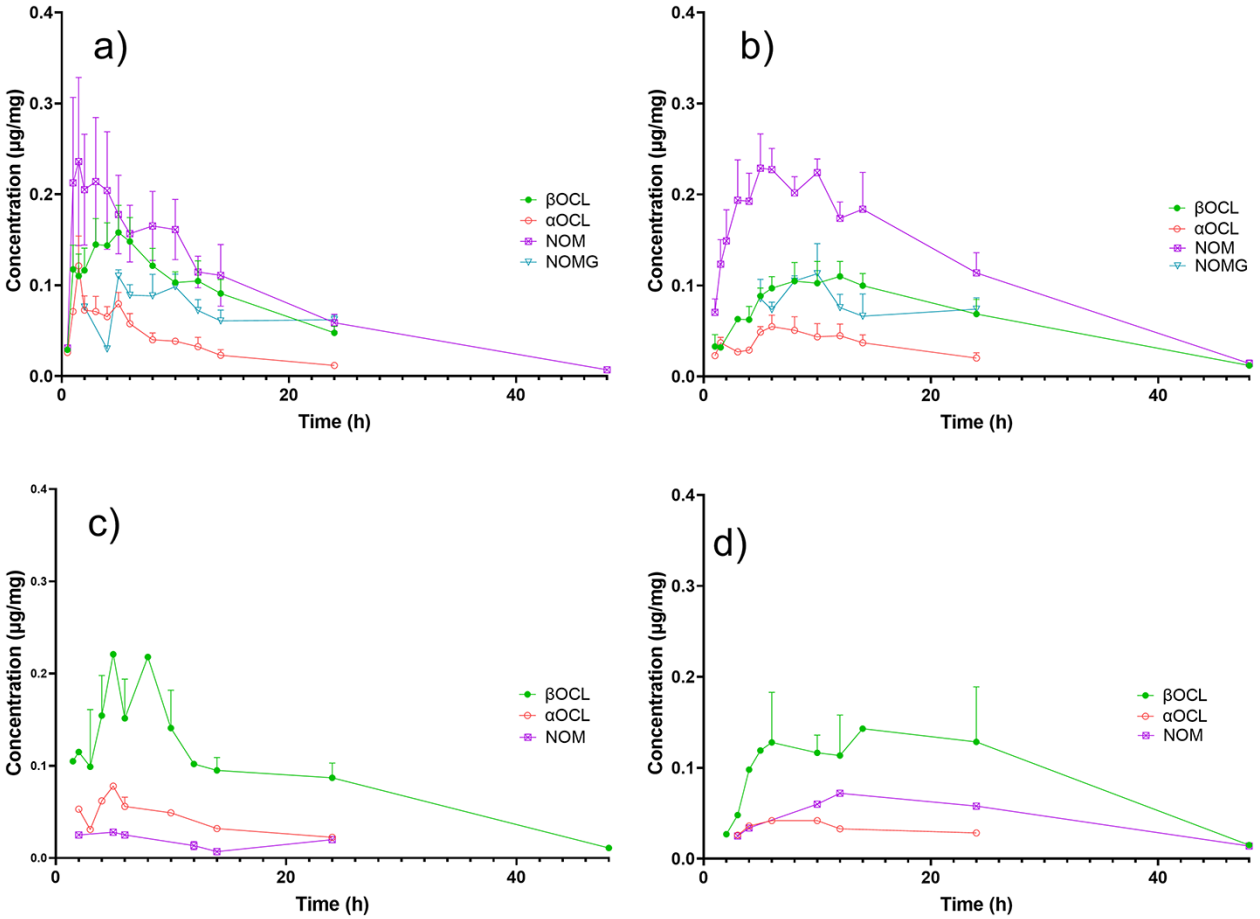

Figure 2. Time–concentration profiles normalized to creatinine after a single dose of 10 mg OC IR and CR of the minor analytes (αOCL, βOCL, NOM and NOMG) grouped by phenotype: (a) IM/EM IR, (b) IM/EM CR, (c) PM IR, (d) PM CR. Data are presented as mean ± standard error of the mean (SEM).

in Figure 1, OC concentrations were greater and peaked earlier after IR compared to CR for IM/EM and PM metabolizers. OMG was detected up to 72 h in all groups except for IM/EM IR. NOC was detected up to 72 h from both IM/EM and PM for CR and up to 48 h for IR for both sets of metabolizers. OC had its shortest detection time (24 h) for IM/EM IR, while it was detectable for up to 48 h for IM/EM CR, PM IR and PM CR. Minor metabolite concentrations were similar for IM/EM metabolizers following IR and CR doses with NOM producing highest concentrations. For PM, NOMG was not detected after either formulation. βOCL had the highest concentrations for the PM after both formulations.

The time-concentration profiles were used to derive the pharmacokinetic data shown in Table II. OM is not included in Table II as it was not detected in sufficient number of subjects. Additionally, the mean detection times for the analytes of interest is also displayed in Table II. Of the major analytes, OMG and NOC had the longest detection time for IR (48 h) and OMG had the longest for CR (65 h). OMG also had the latest T_{max} (8 h) for CR for the major metabolites. For IR, OC had the highest C_{max} (3.4 µg/mg), while NOC had the highest (1.9 µg/mg) for CR. Of the minor analytes, NOM had the longest detection time for IR and CR (29 and 40 h, respectively) and the highest C_{max} (0.18 and 0.23 µg/mg, respectively). When comparing the formulations, OC C_{max} was significantly higher ($P = 0.0329$), detected significantly earlier ($P = 0.03$) and reached maximum concentration earlier ($P = 0.0039$) for IR compared to CR. NOC, αOCL, NOM and OMG reached maximum concentrations significantly earlier ($P = 0.0008$, 0.0221, 0.0119 and 0.0004, respectively) when comparing IR and CR. The detection times for NOC ($P = 0.03$) and OMG ($P = 0.0082$) were significantly longer for CR formulation.

In Figure 3, metabolite ratios (NOC/OC, OMG/OC, βOCL/OC and NOM/OC) are shown grouped by formulation (IR and CR) and by phenotype group (IM/EM and PM). Each metabolite ratio consists of either the major metabolites (NOC and OMG) or the major minor metabolites (βOCL and NOM) compared to parent compound (OC). In general, the metabolite ratios increase over time. There is a steep initial increase, which then plateaus as time progresses. Metabolite ratios were generally lower for PM compared to IM/EM and displayed similar trends within phenotypes compared by formulation. There are fewer points shown for PM ($n = 2$ subjects) because one subject was unable to produce samples at all time points. To correlate the metabolite ratios to the time of intake, linear regressions of the individual ratios for the IM and EM subjects were performed for NOC/OC ($y = 0.5409x$, $R^2 = 0.41$), OMG/OC ($y = 0.2958x$, $R^2 = 0.65$), βOCL/OC ($y = 0.0362x$, $R^2 = 0.74$) and NOM/OC ($y = 0.0502x$, $R^2 = 0.63$) during the first 24 h after intake.

In Figure 4, the predicted time of intake for Subject H are shown for the metabolite to parent ratios NOC/OC,

Table II. Pharmacokinetic Constants Normalized to Creatinine for OC and its Metabolites after Administration of 10 mg CR and IR Tablets of Oxycodone

Major analytes											
Analyte		**OC (IR)**	**OC (CR)**	***P***	**NOC (IR)**	**NOC (CR)**	***P***	**OMG (IR)**	**OMG (CR)**	***P***	
N		7	7		7	7		7	7		
$C_{max} \pm$ SEM (μg/mg)		3.4 ± 0.79	1.3 ± 0.30	0.0329	2.5 ± 0.43	1.9 ± 0.33	0.0686	1.5 ± 0.31	1.1 ± 0.21	0.1136	
$T_{max} \pm$ SEM (h)		2.1 ± 0.64	5.1 ± 0.83	0.0039	3.1 ± 0.73	7.4 ± 1.1	0.0008	3.7 ± 0.64	8.0 ± 1.1	0.0004	
Detection Time + SEM (h)	First	0.93 ± 0.35	1.2 ± 0.31	0.03	1.0 ± 0.35	1.3 ± 0.31	0.1723	1.4 ± 0.30	1.6 ± 0.28	0.3559	
	Last	31 ± 4.4	34 ± 4.9	0.3559	48 ± 0.0	62 ± 4.9	0.03	48 ± 5.2	65 ± 4.4	0.0082	
Minor Analytes											
Analyte		**βOCL (IR)**	**βOCL (CR)**	***P***	**αOCL (IR)**	**αOCL (CR)**	***P***	**NOM (IR)**	**NOM (CR)**	***P***	**NOMG (IR)**	**NOMG (CR)**	***P***
N		7	7		7	7		7	6		5	5	
$C_{max} \pm$ SEM (μg/mg)		0.17 ± 0.02	0.11 ± 0.02	0.0793	0.09 ± 0.02	0.06 ± 0.01	0.0912	0.18 ± 0.06	0.23 ± 0.04	0.6143	0.10 ± 0.01	0.11 ± 0.02	0.7947
$T_{max} \pm$ SEM (h)		4.7 ± 0.42	10 ± 2.5	0.0643	4.2 ± 1.2	6.6 ± 0.81	0.0221	4.4 ± 1.7	8.5 ± 1.7	0.0119	9.0 ± 1.2	9.0 ± 1.5	0.9999
Detection Time + SEM (h)	First	1.5 ± 0.29	3.6 ± 0.81	0.0245	1.7 ± 0.31	3.5 ± 0.72	0.0411	2.9 ± 1.5	1.5 ± 0.34	0.465	4.4 ± 0.68	6.6 ± 1.4	0.1084
	Last	27 ± 3.4	34 ± 4.9	0.1723	22 ± 1.7	24 ± 0.0	0.3559	29 ± 5.0	40 ± 5.1	0.465	18 ± 2.5	22 ± 2	0.1778

Note: OM is not presented.

OMG/OC and βOCL/OC and NOM/OC. The times were calculated using the equations above.

The metabolite ratios from the traffic case were 0.649 for NOC/OC, 0.473 for OMG/OC, 0.208 for βOCL/OC and 0.013 for NOM/OC. Using the determined metabolite ratios and the equations above, the estimated time of intake was calculated. The time of intake was estimated to range from 0.25 to 5.7 h.

Discussion

The purpose of this study was to expand the knowledge of the pharmacokinetics of OC metabolites regarding the excretion of conjugated and minor metabolites, pharmacokinetic differences by formulation and illustrate the impact of CYP2D6 activity.

The detection times of OC were compared between the immunoassay results and the LC–MS-MS results and it was determined that they were comparable to each other. The LOQ for OC for the LC–MS-MS method was 0.015 μg/mL, while the cut off concentration for the immunoassay was 0.1 μg/mL. The average first detection of OC by immunoassay was 1 h after IR administration and 1.4 h for CR. OC was detected up to 38 h for IR and 45 h for CR. This data can benefit the forensic community by demonstrating that using immunoassay can detect OC concentrations from a single administration up to 30+ h depending on the formulation.

The study by Cone et al. involved 12 subjects that were administered a 20 mg CR OC tablet and then monitored hydrolyzed and unhydrolyzed metabolites (OC, NOC, OM and NOM) over 36 h (6). The current study reported concentrations from OC and its metabolites with creatinine correction, while Cone et al. did not. The average creatinine concentration after IR administration was 1.00 mg/mL and 1.08 mg/mL after CR administration. Comparing the current study with the study performed by Cone et al. the C_{max} values for free OC, NOC, NOM and hydrolyzed OM (2.8, 4.4, 0.61 and 1.6 μg/mL) are comparable as they are approximately double the C_{max} values of CR OC, NOC, NOM and OMG (1.3, 1.9, 0.23 and 1.1 μg/mg) from the current study where 10 mg OC was given. The T_{max} of the same analytes between the studies were comparable. A key difference between the studies was the incorporation of conjugated metabolites (OMG and NOMG) into the current study. The minimal detection of OM from the current study is aligned with the results of Cone et al. which reported that OM is primarily excreted in its conjugated form. Additionally, they reported that NOM was excreted in both free and conjugated form but was found predominantly in its free form. This was also discovered from the current study with NOM having higher concentrations than NOMG.

Formulation

The significant differences between IR and CR OC are not unexpected as the formulation is intended to impact release of OC. The CR OC had a lower C_{max} (1.3 μg/mg) and later T_{max} (5.1 h) and first detection (1.2 h) compared to the IR OC. There were no significant differences between the formulations for the last detection of OC. However, NOC and OMG reached maximum concentrations later and were detected longer when comparing IR and CR (14 h longer for NOC and

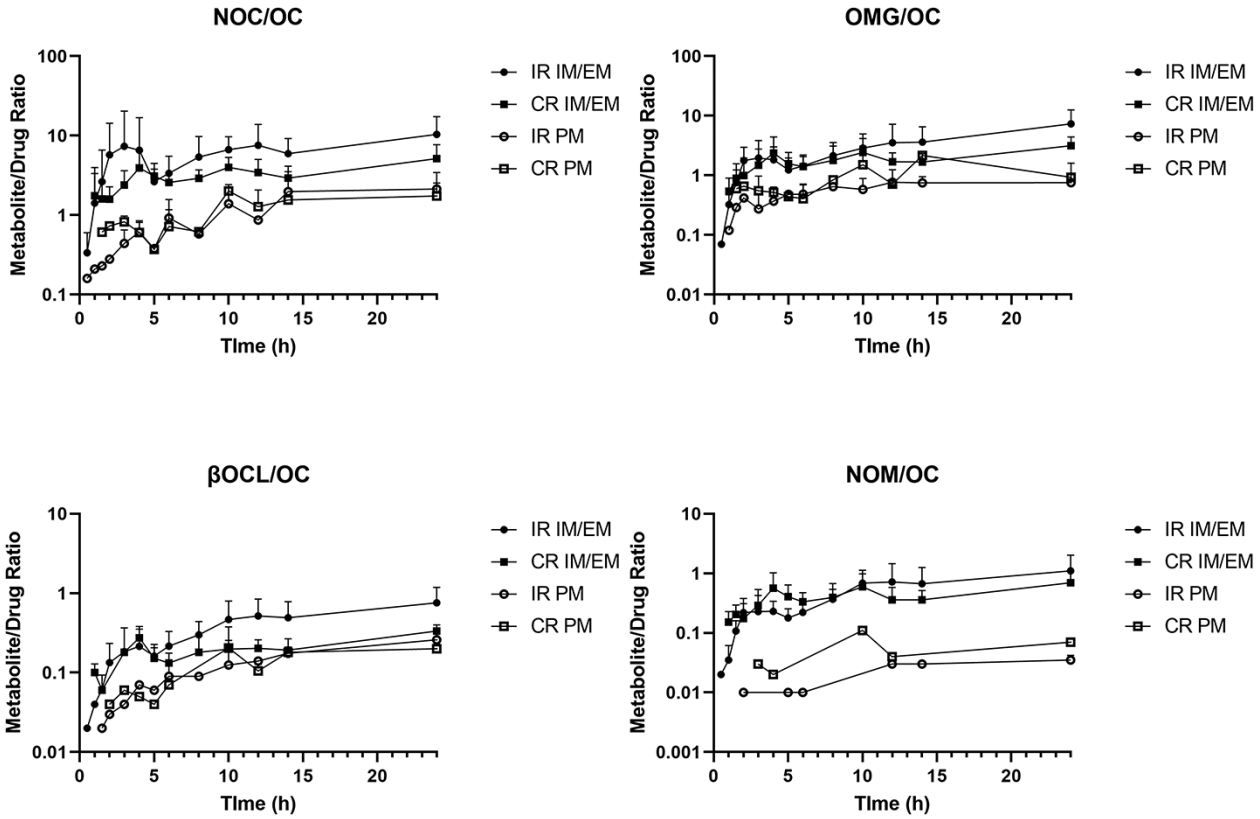

Figure 3. Metabolite ratios for NOC/OC, OMG/OC, βOCL/OC and NOM/OC after a single dose of 10 mg OC IR and CR grouped by phenotype and formulation. Data are present as mean ± standard error of the mean (SEM).

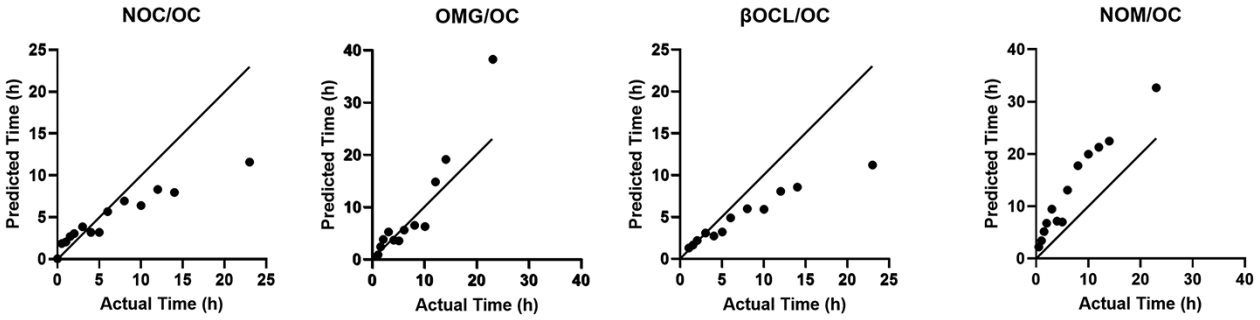

Figure 4. Predicted and actual times of intake estimated from metabolite/parent drug ratios after intake of one 10 mg OC IR tablet. The solid line represents a 100% correlation between predicted and actual sampling time. Predictions were based on the linear regression equations from the study subjects.

17 h longer for OMG). There were no significant differences in maximum concentration and first appearance for NOC and OMG between the two formulations.

Minor metabolites

Of the minor metabolites (αOCL, βOCL, NOM and NOMG) in Figure 2, NOM achieved the highest C_{max} and was detected the longest for both formulations for IM/EM, while for PM it was βOCL that was the primary minor metabolite. Both metabolites were detected up to 48 h regardless of the formulation administered. This difference between the phenotypes is due to CYP2D6 conversion of OC to OM and NOC to NOM which is reflected in PM producing lower NOM concentrations and undetectable NOMG. Production of αOCL and βOCL was not impacted by the phenotype as 6-ketoreductase is the enzyme responsible for the biotransformation. For both formulations and all phenotypes, concentrations for βOCL were higher than its stereoisomer αOCL. Similar findings were reported by Baldacci et al. where capillary electrophoresis was used to analyze oxycodol and noroxycodol stereoisomers (9). It was determined that urinary excretion produced higher amounts of βOCL than αOCL.

Ratios

The most abundant metabolites in major (NOC and OMG) and minor (βOCL and NOM) metabolites were used to generate metabolite/drug ratios. Figure 3 shows a general increase

over time for all four ratios. Even though the number of subjects were small, it was clear that the IM/EM had more metabolites produced as NOC/OC and OMG/OC ratios were >1 within the first 2 h, while the PM subject ratios were <1 until 10 h after administration. Using ratios to estimate the time of intake is difficult unless the predicted phenotype is considered. However, given that only few people are PM a possible general conclusion is that when the NOC/OC ratio is less than unity, the intake was recent. As an example, the data from the subjects were used to predict the time of intake after administration of 10 mg of IR OC. From Figure 4, when using the ratios of OMG and NOM over OC, the time is mainly overestimated, especially in the later time points. On the contrary, for ratios βOCL and NOC over OC, the time is underestimated. From a theoretical viewpoint, using several ratios may help in interpretation. The subject had a normal to slightly lowered metabolic capacity being *1/*41. However, in most authentic cases, the genotype will be unknown to the toxicologist. The case used as an example shows a wide range of predicted times depending on the ratio used (0.25–5.7 h) but supports the claimed intake 3 h prior to sampling.

Limitations

The small number of participants and the predominance of women are the most significant limitations to this study. On the other hand each subject was treated twice, with the IR and ER formulation, enabling a paired *t*-test. In addition, genotyping was performed since the metabolism of OC is sensitive to differences in CYP2D6 activity. Statistical tests were not performed regarding the predicted genotypes but the information was used to illustrate the large interindividual differences in excretion patterns.

Conclusion

The inclusion of NOC and phase II metabolites, especially OMG, expanded the detection window of OC use. There were significant differences between IR and CR for the major metabolites (OC, NOC and OMG) with regards to C_{max}, T_{max} and detection time. The inclusion of the phase II metabolites may help in interpretation and save time with analyses as hydrolysis is not required. There was a general increase in metabolite ratios over time with the parent OC dominating during the first few hours. This change in excretion pattern may be used to estimate a time of intake from the analytical results from a urine sample. Four metabolite to parent ratios were used to predict the time of intake showing that predictions were best at the early time points and that both overestimations and underestimations occurred, depending on the analyte ratio chosen.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Supplementary Data

Supplementary data is available at *Journal of Analytical Toxicology* online.

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