N-Oxyxygenation of Oxycodone and Retro-reduction of Oxycodone N-Oxide

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ABSTRACT

Oxycodone is used as a potent analgesic medication. Oxycodone is extensively metabolized. To fully describe its metabolism, the oxyxygenation of oxycodone to oxycodone N-oxide was investigated in hepatic preparations. The hypothesis tested was that oxycodone N-oxyxygenation was enzymatic and the amount of N-oxide detected was a consequence of both oxyxygenation and retro-reduction. Methods for testing the hypothesis included both in vitro and in vivo studies. Results indicated that oxycodone was N-oxyxygenated by the flavin-containing monooxygenase. Oxycodone N-oxide is chemically quite stable but in the presence of hepatic preparations and NADPH was retro-reduced to its parent compound oxycodone. Subsequently, oxycodone was metabolized to other metabolites including noroxycodone, noroxymorphone, and oxymorphone via cytochrome P-450. Retro-reduction of oxycodone N-oxide to oxycodone was facilitated by quinone reductase, aldehyde oxidase, and hemoglobin but not to a great extent by cytochrome P-450 or the flavin-containing monooxygenase. To confirm the in vitro observations, oxycodone was administered to rats and humans. In good agreement with in vitro results, substantial oxycodone N-oxide was observed in urine after oxycodone administration to rats and humans. Administration of oxycodone N-oxide to rats showed substantial amount of recovered oxycodone N-oxide. In vivo, noroxycodone was formed as a major rat urinary metabolite from oxycodone N-oxide presumably after retro-reduction to oxycodone and oxidative N-demethylation. To a lesser extent, oxycodone, noroxymorphone, and oxymorphone were observed as urinary metabolites.

SIGNIFICANCE STATEMENT

This manuscript describes the N-oxyxygenation of oxycodone in vitro as well as in small animals and humans. A new metabolite was quantified as oxycodone N-oxide. Oxycodone N-oxide undergoes extensive retro-reduction to oxycodone. This re-establishes the metabolic profile of oxycodone and introduces new concepts about a metabolic futile cycle related to oxycodone metabolism.

Introduction

Oxycodone is a potent medication used as an analgesic and antitussive. Oxycodone is also used for severe pain associated with arthritis, disk disease, and cancer (Leow et al., 1992). The potential beneficial properties of oxycodone for pain is somewhat confounded by its incidence of addiction liability. Oxycodone and its metabolite oxymorphone are potent mu opioid receptor agonists (Attila, 1980) and exert their pharmacological activity through this receptor. Oxycodone pharmacokinetics have been studied (Hoskin et al., 1989; Piithiä et al., 1992). Oxycodone is extensively metabolized. Oxycodone is metabolized by cytochrome P450 (P450) enzymes (Guengerich, 2008) to desmethyl metabolites noroxycodone and oxymorphone (Ishida et al., 1982; Baldacci et al., 2004; Lalovic et al., 2004). Once O-demethylated, oxycodols form and can be conjugated in Phase II metabolism.

Tertiary amines such as oxycodone could also in principle be N-oxyxygenated by hepatic flavin-containing monooxygenases (FMOs) (Ziegler, 1990; Cashman, 2002, 2003; Cashman and Zhang, 2006). FMOs accept nucleophilic, lipophilic tertiary amine substrates and generally convert them to polar, tertiary amine N-oxides (Cashman, 2000). In certain cases, prochiral tertiary amines can be stereoselectively N-oxyxygenated by FMO (Cashman et al., 1992; Park et al., 1993). Generally, tertiary amine N-oxides are stable, although in certain cases, Cope-type elimination reactions have been observed (Cashman, 1991). Previously, definitive evidence for metabolic formation of oxycodone N-oxide was not reported. Certain reports suggested the presence of oxycodone N-oxide as a metabolite (Ishida et al., 1979; Moore et al., 2003; Sonar et al., 2012) but no unambiguous evidence was reported.

Tertiary amine N-oxides can also be metabolically retro-reduced to their parent amine (Kitamura et al., 1999). For example, tamoxifen N-oxide is retro-reduced by P450s and hemoglobin (Parte and Kupper, 2005). There was one report of the retro-reduction of aliphatic hydroxylamines by pig FMO1 (Poulsen et al., 1986), but this was not observed for amphetamine or methamphetamine hydroxyamine with human FMO3 (Cashman et al., 1999).

Herein, we report that human liver microsomes form oxycodone N-oxide from oxycodone as determined by LCMS-MS. Oxycodone N-oxide is chemically stable, but in the presence of hepatic microsomes or cytosol, oxycodone N-oxide is rapidly retro-reduced to oxycodone by at least three distinct hepatic protein systems (i.e., quinone reductase, aldehyde oxidase, and hemoglobin). To confirm in vitro observations,

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ABBREVIATIONS: AO, aldehyde oxidase; DETAPAC, diethylenetriaminepentaacetic acid; DPT, dimethylaminodialkyl phenothiazine; FMO, flavin-containing monooxygenase; LCMS-MS, liquid chromatography tandem mass spectrometry; MMI, N-methyl mercapto imidazole; P450, cytochrome P450; SAR, structure activity relationship.

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Oxycodone was administered to rats and humans. In both cases, significant amounts of oxycodone N-oxide was formed. Administration of oxycodone N-oxide to rats showed significant amounts of urinary oxycodone and its metabolites and confirmed retro-reduction in vivo.

**Methods**

**General**

Reagents, starting materials, and solvents were purchased in the highest purity available from commercial suppliers and used as received. Human clinical grade oxycodone, oxycodone N-oxide, d6-oxycodone, oxymorphone, noroxycodone, noroxymorphone, and (α and β)-oxycodols in >99% pure (based on good laboratory practice certificates of analysis) were obtained from Noramco (Athens, GA). Morphine-6-glucuronide was obtained from the National Institute on Drug Abuse drug repository (National Institutes of Health). Naltrexone was purchased from Mallincrodt (Bedminster, NJ). Trimethylamine, 2-mercapto-1-methylimidazole (MMI), cotinine, nicotine, menadione, KCN, sodium arsenite, hemoglobin, alpha naphthylflavone, quinidine, NADP⁺, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and other components of the NADPH-generating system were commercially available from VWR (San Diego, CA). Troleandomycin (TAO) was from Enzo Biochemical, Inc. (Farmingdale, NY). N,N-Dimethyl-alkyl-phenothiazines (i.e., 3-DPT, 5-DPT, and 8-DPT) were synthesized as reported previously (Brunelle et al., 1997). Compounds were tested as hydrochloride salts unless otherwise noted. Phosphate buffer, diethylenetriaminepentaacetic acid, catalase, and other reagents in the microsome or highly purified enzyme assays were purchased from Life Technologies (Carlsbad, CA).

**Photometric Incubations.** Human liver microsomes and CYP1A1, 2A6, 2D6, 3A4 were purchased from BD Gentest (Corning, Corning, NY). Human liver microsomes possessed FMO3 activity [e.g., HH18 or HK37, 2800 and 3600 pmol of methyl p-tolyl sulfide S-oxide formed per (milligram protein × minute)], respectively. Human liver microsomes possessed P450 activity (e.g., HH18 and HK37, 12,000 and 2500 pmol of 6β-hydroxy testosterone formed per (milligram protein × minute), respectively. Human liver S-9 was from Cellz Direct (Durham, NC). Human liver cytosol was from Xenotech (Kansas City, KS). Highly purified mouse FMO1, FMO3, and FMO5 was prepared as described previously (Brunelle, et al., 1997, Motika, et al., 2012). A typical photometric incubation mixture contained 5–10 µg recombinant FMO protein and NADPH (0.2 mM) in 0.05 M potassium phosphate buffer (pH 7.4 or pH 10) containing 0.3 mM diethylenetriaminepentaacetic acid (DETAPAC, as an anti-oxidant) placed on ice. Test compounds (10–100 µM) were added to initiate the incubations at 37°C. The

![Fig. 1. Metabolic scheme for phase I metabolism of oxycodone and metabolites.](#)
consumption of NADPH was monitored by ultraviolet-vis and initial rate measurements obtained. Time- and protein-dependent studies were done to establish conditions for optimal substrate-dependent studies.

**Other Incubations.** A typical incubation was conducted in a total volume of 0.25 ml. Incubation mixtures contained 0.4 mg protein, an NADPH-generating system consisting of NADP⁺ (0.5 mM), glucose-6-phosphate (0.5 mM), glucose-6-phosphate dehydrogenase (1 IU), diethylenetriaminepentaacetic acid (0.3 mM, DETAPAC, as an anti-oxidant), and 0.05 M potassium phosphate buffer (pH 7.4 or pH 10) placed on ice. Incubations were initiated by the addition of test compounds to the ice-cold incubation and placed at 37°C with constant shaking. Incubations were stopped by flash-freezing in dry ice with or without the addition of 0.5 ml 0.5% ammonium hydroxide. Before LCMS analysis, a cold acetonitrile solution containing an internal standard (d⁶-oxycodone) was added to the sample or calibration standard. For anaerobic incubations, screw cap culture tubes were used and microscopic incubations were bubbled with argon gas and sealed under an atmosphere of Argon.

**Analytical.** Prior to analysis, frozen incubations were combined with 0.5% ammonium hydroxide in acetonitrile (0.5 ml), thawed, mixed thoroughly, and centrifuged (15,000 rpm, 7 minutes). Analysis of selected in vitro samples was conducted using a multiple reaction monitoring/ information dependent acquisition/enhanced product ion analysis method. The aqueous-organic material (20 μl) was reconstituted for LC/MS/MS analysis. The mass spectrometer was an Applied Biosystems 4000 QTrap® with a Turbo Spray source (Applied Biosystems, Waltham, MA). LC pumps were Shimadzu/LC-10AD VP (Shimadzu, Columbia, MD). LC was run on a RP column (i.e., Thermo BetaBasic phenyl 3 μm, 150 × 2.1 mm i.d.) equilibrated with 93% Solvent A (0.02 M ammonium formate, 0.2% formic acid in water) and 7% solvent B (0.2% formic acid in acetonitrile/isopropanol (95:5)) and the eluant was detected by electrospray ionization mass spectrometry (ESI MS). Typical LCMS retention times for this system were: noroxymorphone (2.35 minutes), oxymorphone (2.72 minutes), noroxycodone (4.81 minute), d⁶-oxycodone (5.37 minute), oxycodone (5.44 minute), oxycodone-N-oxide (6.86 minutes) and were all simultaneously analyzed by ESI LCMS-MS via multiple reaction monitoring using d⁶-oxycodone as an internal standard.

**Heat Inactivation Studies**

Microsomes were placed in buffer and DETAPAC (0.3 mM) and 2 IU of catalase in a screw cap culture tube and purged with argon gas, sealed with a Teflon screw-tight cap placed on ice. The incubation tube was placed in ice, the cap removed and the NADPH-generating system was added. The incubation was initiated by the addition of substrate at 37°C with shaking. Generally, under these conditions 60%–85% of FM03 activity was lost while at the same time decreasing P450 only marginally (Cashman, 1999).

**Aldehyde Oxidase or Quinone Reductase Assays**

Cotinine formation was a convenient method for verifying hepatic aldehyde oxidase (AO) formation. Conversion of CYP2A6-generated nicotine iminium ion to cotinine was used to determine AO activity (Cashman et al., 1992). Incubations were as above but human liver cytosol or human liver S-9 (0.5 mg protein) was used in the presence of Tris buffer (50 mM, pH 7.4) containing 3 mM MgCl₂. At the termination of the incubation, cold dichloromethane (1 ml) and sodium carbonate (10 mg) was added followed by thorough mixing and centrifugation (4000 rpm). The organic layer was separated, evaporated to dryness, and reconstituted in MeOH for analysis of nicotine and cotinine (Cashman et al., 1992). Quinone reductase assays were conducted as above. For anaerobic incubations, screw cap culture tubes were used and cytosolic incubations were bubbled with argon gas and sealed under an atmosphere of argon.

**Data Analysis**

Statistics including mean, S.D., relative S.D., and linear regression analysis where conducted when appropriate. Values were compiled using Microsoft Excel and Prism software.

**Animal Studies**

Animal work followed the Guide for Care and Use of Animals as adopted by National Institutes of Health. Formal approval was obtained from the Institutional Animal Care and Use Committee of the Human BioMolecular Research Institute, San Diego, CA. Five male and five female 7 week-old rats (Charles River, Hollister, CA) were administered compounds (oral) and placed in stainless steel metabolic chambers. Urine was collected and food and water consumption was monitored. Two animals of each sex were administered oxycodone N-oxide (20 mg/kg, oral) in sterile water. Three animals of each sex were administered oxycodone HCl (20 mg/kg, oral) in sterile water.

**Human Studies**

Urine for quantification of oxycodone, noroxycodone, oxymorphone, and oxycodone N-oxide was collected as part of a Phase 1, three-way crossover, active controlled, pharmacokinetic study to determine the pharmacokinetics of PTI-821, a slow release form of oxycodone, in healthy volunteers. The study protocol was approved by IntegReview IRB (Austin, TX). Subjects underwent an overnight 10-hour fast prior to drug administration. Subjects received an oral dose of 50 mg naltrexone HCl the evening prior to as well as 30 minutes prior to drug administration. After administration of 40 mg oxycodone, urine was collected from 0 to 12, 12 to 24, 24 to 36, 36 to 48, 48 to 60, 60 to 72, 72 to 84, and 94 to 96 hours after dosing. Urine data were used to calculate the total amount of each analyte excreted in the urine and to estimate the percentage of the administered dose of oxycodone recovered in the urine. Only subjects with complete urine collection through 96 hours were included in the calculations. After collection, urine was frozen and shipped to Advion BioServices, Inc. (Ithaca, NY) for bioanalysis.
For comparison, we also examined \(N\)-philic tertiary amines and converts them to polar, readily excreted \(N\) and 20.2 (nmol/min)/mg of protein. Oxycodone and oxymorphone and FMO5 (see Table 1). Recombinant FMO enzyme was expressed amine opioids with highly purified recombinant mouse FMO1, FMO3, and FMO5 (see Table 1). Recombinant FMO enzyme was expressed and purified to near homogeneity and used in photometric assays (i.e., NADPH consumption). For FMO3, oxygenation of prototypical substrates MMI and trimethyleneamine occurred at normal rates [i.e., 18.3 and 20.2 (nmol/min)/mg of protein]. Oxycodone and oxymorphone showed even greater specific rates of \(N\)-oxygenation (Table 1).

### Results

For oxycodone, microsomal metabolism studies showed that in addition to metabolites previously reported formed (i.e., oxymorphone, noroxycodone, and noroxymorphone), oxycodone \(N\)-oxide was also observed (Fig. 1). After administration of oxycodone, we also found oxycodone \(N\)-oxide in the urine of rats and relatively high levels in the urine of humans. Accordingly, we examined the \(N\)-oxygenation of oxycodone and other related compounds in some detail.

### In Vitro N-Oxygenation with FMOs.

FMO \(N\)-oxygenates nucleophilic tertiary amines and converts them to polar, readily excreted tertiary amine N-oxides (Cashman, 2004). We examined the \(N\)-oxygenation of selected tertiary amine opioids, metabolites, and prototypical substrates with highly purified human FMO3 and FMO5. For comparison, we also examined \(N\)-oxygenation of selected tertiary amine opioids with highly purified recombinant mouse FMO1, FMO3, and FMO5 (see Table 1). Recombinant FMO enzyme was expressed and purified to near homogeneity and used in photometric assays (i.e., NADPH consumption). For FMO3, oxygenation of prototypical substrates MMI and trimethyleneamine occurred at normal rates [i.e., 18.3 and 20.2 (nmol/min)/mg of protein]. Oxycodone and oxymorphone showed even greater specific rates of \(N\)-oxygenation (Table 1).

### Table 3

<table>
<thead>
<tr>
<th>Human Liver Microsome</th>
<th>Time</th>
<th>Oxycodone</th>
<th>Oxycodone N-Oxide</th>
<th>Noroxycodone</th>
<th>Oxymorphone</th>
<th>Noroxymorphone</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK37(a)</td>
<td>0</td>
<td>197.45 ± 5.16</td>
<td>0.20 ± 0.11</td>
<td>0.25 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.011 ± 0.015</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>196.95 ± 7.14</td>
<td>0.33 ± 0.003</td>
<td>1.73 ± 0.05</td>
<td>0.67 ± 0.05</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>195.45 ± 7.14</td>
<td>0.36 ± 0.07</td>
<td>1.70 ± 0.03</td>
<td>0.63 ± 0.04</td>
<td>0.013 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>186.60 ± 7.66</td>
<td>0.29 ± 0.05</td>
<td>0.69 ± 0.08</td>
<td>0.06 ± 0.003</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>163.70 ± 5.07</td>
<td>0.29 ± 0.07</td>
<td>1.69 ± 0.35</td>
<td>0.13 ± 0.01</td>
<td>ND</td>
</tr>
</tbody>
</table>

**ND, not detected.**

\(a\) CYP3A4 activity was 2.5 (nmol of product/min)/mg protein in the testosterone 6\(\beta\)-hydroxylase assay. FMO activity was 3.6 (nmol of product/min)/mg protein in the methyl \(p\)-tolyl sulfide oxidase assay.

### Table 4

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</tr>
</thead>
<tbody>
<tr>
<td>HK37(a)</td>
<td>0</td>
<td>210.50 ± 1.70</td>
<td>0.32 ± 0.001</td>
<td>0.42 ± 0.09</td>
<td>0.04 ± 0.01</td>
<td>0.013 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>196.70 ± 10.61</td>
<td>0.63 ± 0.01</td>
<td>5.08 ± 0.18</td>
<td>0.97 ± 0.02</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>205.35 ± 0.64</td>
<td>0.70 ± 0.05</td>
<td>5.89 ± 0.11</td>
<td>1.12 ± 0.06</td>
<td>0.09 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>166.03 ± 4.08</td>
<td>0.27 ± 0.05</td>
<td>0.45 ± 0.09</td>
<td>0.02 ± 0.004</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>170.33 ± 9.08</td>
<td>0.37 ± 0.08</td>
<td>1.07 ± 0.12</td>
<td>0.07 ± 0.004</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>163.73 ± 6.25</td>
<td>0.32 ± 0.001</td>
<td>0.42 ± 0.09</td>
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### References

CYP3A4 activity was 12.0 (nmol of product/min)/mg protein in the testosterone 6\(\beta\)-hydroxylase assay. FMO activity was 2.8 (nmol of product/min)/mg protein in the methyl \(p\)-tolyl sulfide oxidase assay.

Interestingly, neither the N-cyclopropyl methyl opioid, naltrexone, nor the 6-glucuronide of morphine (i.e., M-6-G) were detectably \(N\)-oxygenated by human FMO3. As discussed previously (Ziegler, 1990; Cashman, 2000), FMO3 is sensitive to steric bulk around the tertiary amine moiety, and it is not surprising that naltrexone was not a good substrate for human FMO3. The polar nature of the glucuronide, morphine-6-glucuronide, probably precluded it as a substrate for FMO3. Highly purified mouse FMO \(N\)-oxygenated oxymorphone and oxycodone (Table 1). Somewhat surprisingly, naltrexone was \(N\)-oxygenated by mouse FMO. It may be that mouse FMO3 tolerates substrates with larger steric bulk around the tertiary amine. Mouse FMO1 also \(N\)-oxygenated all three substrates. Unfortunately, an active hFMO1 prep was not available for comparison. The data were in agreement with the literature that suggested FMO1 has a slightly more accommodating substrate binding domain than FMO3 (Ziegler, 1990; Cashman, 2000). The \(N\)-oxygenation of oxycodone, oxymorphone, and naltrexone by mouse and human FMO5 was examined. Little has been reported on the \(N\)-oxygenation of oxycodone, oxymorphone, and naltrexone by mouse and human FMO5. In contrast to human FMO5, mouse FMO5 did not \(N\)-oxygenate naltrexone. The similarity of mouse and human FMOs to \(N\)-oxygenate the tertiary amine was striking. We previously reported that tertiary amines were substrates for mouse FMO5, and these data are in agreement with that report (Motika et al., 2012). Overall, the data are in agreement with reports that show a reasonable agreement between the \(N\)-oxygenation of selective functional substrates by mouse and human FMOs (Motika et al., 2012). These data are shown in Table 2. Table 2 shows that mouse and human FMOs show a similar profile of \(N\)-oxygenation for selective functional substrates of FMO1, 3, and 5. It is
notable that, compared with human FMO1, mouse FMO1 more efficiently N-oxygenated 3-, 5-, and 8-DPT. This was confirmed by a comparison of the catalytic efficiency (Vₘₐₓ/Kₘₐₓ) of MMI for mouse FMO1 (3.9) and human FMO1 (0.4), affording about a 10-fold difference. This is likely due to the stability and inherent robustness of FMO1 (3.9) and human FMO1 (0.4), affording about a 10-fold difference. This is likely due to the stability and inherent robustness of FMO1 (3.9) and human FMO1 (0.4), affording about a 10-fold difference.

Microsomal Metabolism of Oxycodone. In the presence of human liver microsomes enriched with FMO3 (preparation HK37), pH 7.4, and supplemented with NADPH, oxycodone (200 μM) was converted to noroxycodone and oxymorphine. Significant amounts of oxycodone N-oxide were observed but no noroxymorphone was detected (Table 3). The time course for product formation was quite rapid and linear for only 10 minute.

To explore a possible role of FMO3 in metabolite formation, incubations were repeated at pH 10. In contrast to P450 (pH optima of 7.4), FMO3 has a pH optima of 10 (Cashman, 2005). In the presence of human liver microsomes enriched with FMO3 (pH 10), noroxycodone and oxymorphine formation was significantly increased 3- and 1.5-fold, respectively, compared with incubations run at pH 7.4. At pH 10, oxycodone N-oxide was increased almost 2-fold but oxymorphine formation was not detected (Table 4). In contrast, in the presence of human liver microsomes (pH 10) enriched in CYP3A4, less noroxycodone and oxymorphine were formed (Table 4). Again, no detectable amount of noroxymorphone was observed. As expected, compared with incubations at pH 7.4, an increase in formation of oxycodone N-oxide was observed at pH 10 for both microsome preparations, but microsomes enriched in FMO3 showed the greatest N-oxide product formation (Table 4).

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Microsomal Metabolism of Oxycodone. In the presence of human liver microsomes enriched with FMO3 (preparation HK37), pH 7.4, and supplemented with NADPH, oxycodone (200 μM) was converted to noroxycodone and oxymorphine. Significant amounts of oxycodone N-oxide were observed but no noroxymorphone was detected (Table 3). The time course for product formation was quite rapid and linear for only 10 minute.

To explore a possible role of FMO3 in metabolite formation, incubations were repeated at pH 10. In contrast to P450 (pH optima of 7.4), FMO3 has a pH optima of 10 (Cashman, 2005). In the presence of human liver microsomes enriched with FMO3 (pH 10), noroxycodone and oxymorphine formation was significantly increased 3- and 1.5-fold, respectively, compared with incubations run at pH 7.4. At pH 10, oxycodone N-oxide was increased almost 2-fold but oxymorphine formation was not detected (Table 4). In contrast, in the presence of human liver microsomes (pH 10) enriched in CYP3A4, less noroxycodone and oxymorphine were formed (Table 4). Again, no detectable amount of noroxymorphone was observed. As expected, compared with incubations at pH 7.4, an increase in formation of oxycodone N-oxide was observed at pH 10 for both microsome preparations, but microsomes enriched in FMO3 showed the greatest N-oxide product formation (Table 4).
When the study was repeated at 200 μM oxycodone, noroxycodone, and oxymorphone were observed (Table 9). With incubations done at pH 7.4, in the presence of oxycodone N-oxide, we observed retro-reduction, the study was repeated at pH 10. Compared to catalyze the apparent retro-reduction, the study was repeated at pH 10 (the pH optima for FMO3). This result was in agreement with the data of Table 1 showing oxycodone was a substrate for human FMO3. An extremely small amount of noroxycodone was also detected in the incubation with human FMO3 but no oxymorphone or noroxymorphone was observed. Because certain tertiary amine N-oxides are unstable (Cashman, 1991), we examined the chemical and metabolic stability of oxycodone N-oxide in the presence of microsomes and purified enzymes.

**Highly Purified FMO3.** The ability of highly purified human FMO3 to catalyze the N-oxygenation of oxycodone was examined. As shown in Table 7, oxycodone was N-oxygenated by human FMO3 supplemented with NADPH. This result was in agreement with the data of Table 1 showing oxycodone was a substrate for human FMO3. An extremely small amount of noroxycodone was also detected in the incubation with human FMO3 but no oxymorphone or noroxymorphone was observed. Because certain tertiary amine N-oxides are unstable (Cashman, 1991), we examined the chemical and metabolic stability of oxycodone N-oxide in the presence of microsomes and purified enzymes.

**Studies with Oxycodone N-Oxide.** Incubation of oxycodone N-oxide in phosphate buffer over extended periods of time (24–48 hours) showed no indication of degradation as judged by HPLC. We concluded oxycodone N-oxide was sufficiently stable for metabolism studies. In the presence of human liver microsomes enriched with FMO3 (pH 7.4), a very fast conversion of oxycodone N-oxide (200 μM) to its retro-reduced metabolite oxycodone was observed (Table 8). Significant amounts of noroxycodone and oxymorphone were also formed. However, in the presence of a lower oxycodone N-oxide concentration (20 μM), an even greater percent conversion of oxycodone N-oxide to oxycodone and noroxycodone was observed (Table 8). In the presence of low oxycodone N-oxide substrate conditions, no oxymorphone or noroxymorphone was detected (Table 8). To examine this point further, we investigated the retro-reduction of oxycodone N-oxide in vitro (Fig. 1). Because of the apparent rapid oxycodone N-oxide retro-reduction and oxycodone metabolism observed, N-oxygenation was likely underestimated both in human liver microsomes and other hepatic preparations. To explore this point further, we investigated the retro-reduction of oxycodone N-oxide.

The conversion of oxycodone N-oxide to oxycodone in the presence of human liver microsomes, S-9, and purified enzymes was examined to identify the reductase(s) responsible for retro-reduction. Incubations were done under anaerobic conditions and aerobic conditions. As shown in Table 8, in the presence of human liver microsomes, anaerobic retro-reduction of oxycodone N-oxide was observed (pH 7.4). The ratio of oxymorphone formed versus oxycodone N-oxide (53% vs. 41%) was not strongly dependent on the presence of FMO3. The ratio of noroxymorphone formed versus oxycodone N-oxide (6.5% vs. 7%) was also not strongly dependent on the presence of cytochrome P450 or FMO3. To further examine a role of quinine reductase and other hemoprotein systems in the retro-reduction of oxycodone N-oxide, we observed retro-reduction of oxycodone N-oxide in vitro (Fig. 1).

The conversion of oxycodone N-oxide to oxycodone in the presence of human liver microsomes, S-9, and purified enzymes was examined to identify the reductase(s) responsible for retro-reduction. Incubations were done under anaerobic conditions and aerobic conditions. As shown in Table 8, in the presence of human liver microsomes, anaerobic retro-reduction of oxycodone N-oxide was observed (pH 7.4). The ratio of oxymorphone formed versus oxycodone N-oxide (53% vs. 41%) was not strongly dependent on the presence of FMO3. The ratio of noroxymorphone formed versus oxycodone N-oxide (6.5% vs. 7%) was also not strongly dependent on the presence of cytochrome P450 or FMO3. To further examine a role of either cytochrome P450 or FMO3 in oxycodone N-oxide retro-reduction, the incubation was repeated at pH 10 (the pH optima for FMO3).

<table>
<thead>
<tr>
<th>Human Liver Microsomes</th>
<th>Oxycodone</th>
<th>Oxycodone N-Oxide</th>
<th>Noroxycodone</th>
<th>Oxydorphine</th>
<th>Noroxymorphone</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK37*</td>
<td>48.6 ± 3.1</td>
<td>115.9 ± 5.8</td>
<td>7.5 ± 0.5</td>
<td>0.2 ± 0.01</td>
<td>ND</td>
</tr>
<tr>
<td>HH18*</td>
<td>5.0 ± 0.2</td>
<td>9.4 ± 0.5</td>
<td>0.6 ± 0.02</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Oxycodone N-oxide was present at 200 μM.

**ND, not detected.**

### Table 7

Concentration of oxycodone metabolites in the presence of purified FM03 samples incubated with 200 μM oxycodone HCl at pH 7.4

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Oxycodone (nmol/ml)</th>
<th>Oxycodone N-Oxide (nmol/ml)</th>
<th>Noroxycodone (nmol/ml)</th>
<th>Oxydorphine (nmol/ml)</th>
<th>Noroxymorphone (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>152.88 ± 8.10</td>
<td>2.09 ± 0.68</td>
<td>0.008 ± 0.002</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not detected.
As shown in Table 9, the ratio of oxycodone formed versus oxycodone N-oxide (29% vs. 74%) after 20 minute was greater at pH 10 in the presence of microsome samples enriched in P450. The ratio of noroxycodone formed versus oxycodone N-oxide (4.4% vs. 11%) after 20 minute was also greater at pH 10 in the presence of microsomes enriched in P450. We interpreted this result to suggest both P450 and FMO3 may be contributing to microsomal retro-reduction. To explore this point, heat inactivation studies were done.

As shown in Table 10, the ratio of oxycodone formed versus oxycodone N-oxide (22% vs. 38%) after a 20-minute incubation was less in the presence of heat-inactivated microsome samples enriched in FMO3 at pH 7.4. The ratio of noroxycodone formed versus oxycodone N-oxide (3.5% vs. 5.6%) after 20 minutes was also less in the presence of microsomes enriched in FMO3. Highly purified human FMO3 did not have a marked effect on the retro-reduction of oxycodone N-oxide and the alternate competitive substrate, MMI, did not have any apparent effect on retro-reduction (data not shown). The results suggested that FMO3 may have contributed to microsomal retro-reduction, but the contribution was very minor or noncatalytic and relatively rapid based on the amount of product formed at the zero time point. To confirm this, oxycodone N-oxide was incubated with highly purified human FMO3 at pH 7.4 (Table 11). Both oxycodone and noroxycodone were detected, albeit at low levels. Accordingly, we examined for the involvement of other retro-reduction systems in human liver microsomes.

Retro-reduction of oxycodone N-oxide in human liver microsomes was strongly dependent on NADPH (Table 12). Compared with the complete system, in the absence of NADPH or in the absence of NADPH plus menadione, inhibition of retro-reduction to oxycodone was 93% and 92%, respectively (Table 12). Likewise, compared with the complete system, in the absence of NADPH or in the absence of NADPH plus menadione, inhibition of formation of noroxycodone was 95% and 98%, respectively. In the presence of menadione alone, inhibition of retro-reduction to oxycodone N-oxide was 73%. In the presence of menadione alone, inhibition of formation of noroxycodone was 95%. The results suggested a contribution from quinone reductase in the conversion of oxycodone N-oxide to oxycodone because: 1) anaerobic retro-reduction of oxycodone N-oxide was NADPH dependent, and 2) retro-reduction activity was not enhanced by other quinones (Kitamura et al., 1999).

Another reductase system present in cytosolic hepatic preparations is aldehyde oxidase (AO) (Pryde et al., 2010). Menadione is a potent inhibitor of AO and is used to eliminate AO so other reductases can be detected. The study was repeated with human liver cytosol (20 μM, pH 7.4, 30 minutes) that had been characterized for AO functional activity. The results paralleled the results observed in human liver microsomes although less dramatic. To study this aspect in greater detail, human liver S-9 was examined, because it contained both cytosol and components that comprise microsomes.

**Oxycodone N-Oxide Retro-reduction in the Presence of S-9.** As shown in Table 13, in the presence of S-9, oxycodone N-oxide retro-reduction was not inhibited by KCN or sodium arsenite. Addition of hemoglobin markedly increased the rate of oxycodone N-oxide retro-reduction and noroxycodone formation. The results suggested a contribution from quinone reductase and other hemoproteins such as hemoglobin in the retro-reduction of oxycodone N-oxide. We examined other hemoproteins such as highly purified P450s (i.e., human CYP1A1, CYP2A6, CYP2D6, and CYP3A4) supplemented with NADPH in the reduction of oxycodone N-oxide.

**Metabolism of Oxycodone N-Oxide by Highly Purified CYPs and FMO3.** cDNA-expressed human CYP1A1, CYP2A6, CYP2D6, CYP3A4, and FMO3 were examined for their ability to retro-reduce oxycodone N-oxide (Table 14). In general, robust P450-mediated retro-reduction of oxycodone N-oxide was not observed. NADPH alone did not retro-reduce oxycodone N-oxide, suggesting that reducing equivalents alone were not sufficient to support reduction of the N-oxide. Based on use of selective functional inhibitors, CYP1A1 and CYP2D6 may have contributed to slight retro-reduction of oxycodone N-oxide but the amount of retro-reduction was very small. In

### TABLE 9

<table>
<thead>
<tr>
<th>Human Liver Microsome</th>
<th>Time (min)</th>
<th>Oxycodone</th>
<th>Oxycodone N-Oxide</th>
<th>Noroxycodone</th>
<th>Oxymorphone</th>
<th>Noroxymorphone</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>38.8 ± 2.2</td>
<td>147.9 ± 2.5</td>
<td>5.3 ± 0.3</td>
<td>0.1 ± 0.001</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>42.7 ± 4.5</td>
<td>164.6 ± 14.6</td>
<td>6.4 ± 0.8</td>
<td>0.2 ± 0.04</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>42.6 ± 1.9</td>
<td>142.4 ± 8.3</td>
<td>6.2 ± 0.04</td>
<td>0.2 ± 0.01</td>
<td>ND</td>
</tr>
<tr>
<td>HH18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>6.5 ± 0.8</td>
<td>13.3 ± 2.2</td>
<td>9.9 ± 0.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6.8 ± 0.6</td>
<td>12.5 ± 2.2</td>
<td>1.0 ± 0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>9.4 ± 0.7</td>
<td>12.6 ± 5.5</td>
<td>1.4 ± 0.3</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not detected.
<sup>a</sup>Oxycodone N-oxide was present at 200 μM.
<sup>b</sup>Oxycodone N-oxide was present at 20 μM.

### TABLE 10

<table>
<thead>
<tr>
<th>Human Liver Microsomes HK37&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time</th>
<th>Oxycodone</th>
<th>Oxycodone N-Oxide</th>
<th>Noroxycodone</th>
<th>Oxymorphone</th>
<th>Noroxymorphone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>(nmol/ml/mg protein)</td>
<td>(nmol/ml)</td>
<td>(nmol/ml/mg protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No heat treatment</td>
<td>0</td>
<td>2.5 ± 0.2</td>
<td>11.8 ± 0.5</td>
<td>0.3 ± 0.02</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.4 ± 0.2</td>
<td>11.3 ± 2.0</td>
<td>0.6 ± 0.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>0</td>
<td>2.9 ± 0.4</td>
<td>12.0 ± 1.3</td>
<td>0.4 ± 0.06</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.5 ± 0.3</td>
<td>15.8 ± 5.2</td>
<td>0.6 ± 0.2</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not detected.
<sup>a</sup>Oxycodone N-oxide was present at 20 μM.
the presence of alpha naphthyllflavone, apparent inhibition of CYP1A1-mediated reduction of oxycodone N-oxide was observed. Similarly, in the presence of quinidine, apparent inhibition of CYP2D6-mediated oxycodone N-oxide reduction was observed. The FMO alternate substrate competitive inhibitor MMI had no apparent effect on oxycodone N-oxide retro-reduction (Table 14). The overall contribution of CYP1A1, CYP2D6, or FMO3 to oxycodone N-oxide retro-reduction was very small in comparison with other reductase systems. In the presence of CYP1A1, CYP2A6, CYP2D6, CYP3A4, and FMO3, no other products (i.e., noroxycodone, oxymorphone, or noroxymorphone) were formed from oxycodone N-oxide (Table 14).

**Oxycodone Administration to Rats: Oxidation of Oxycodone In Vivo.** After administration of oxycodone (20 mg/kg, oral) to rats the urinary metabolic profile showed that oxycodone was converted to oxycodone N-oxide, noroxycodone, oxymorphone, and noroxymorphone as determined by LCMS-MS. The amount of oxycodone N-oxide recovered in the urine was relatively modest. In male rats, the amount of oxycodone N-oxide excreted over 24 hours was approximately 0.14% of the total mass of parent oxycodone and characterized metabolites excreted. In female rats, the amount of oxycodone N-oxide excreted over 24 hours was approximately 2% of the total mass of parent oxycodone and characterized metabolites excreted. The sex difference may be due to the fact that the amount of FMO3 in young male rats is much lower than that in young female rats (Lattard et al., 2001, 2002).

**Oxycodone N-Oxide Administration to Rats In Vivo.** After administration of oxycodone N-oxide (20 mg/kg, oral), a large amount of unchanged oxycodone N-oxide was observed in both male and female rats. The predominant metabolite by far was noroxycodone, followed in descending order by oxycodone, noroxymorphone, and oxymorphone. Retro-reduction of oxycodone N-oxide to oxycodone was efficient as evidenced by a significant amount of oxycodone and oxycodone metabolites present in the urine.

**Oxycodone Administered to Humans.** In humans, a total of 9.6 mg of oxycodone and metabolites were recovered in urine after administration of 40 mg PTI-821 (a slow release form of oxycodone). The order of prevalence and percent recovery based on total free drug and metabolites excreted (i.e., 27.3%) included noroxycodone (66%) followed by intact oxycodone (27.1%), oxycodone N-oxide (5.5%), and oxymorphone (0.77%) as determined by LCMS-MS. The greatest amount of oxycodone and metabolites recovered was during the first 12 hours of urine collection.

**Discussion**

P450-dependent O- and N-demethylation are well-recognized metabolic processes (Guengerich, 2008). Oxycodone is converted to its N-desmethyl metabolite, noroxycodone, by the action of oxycodone O-demethylase. O-Demethylation is also involved in the conversion of oxycodone to oxymorphone. Both oxymorphone and noroxycodone are metabolized to noroxymorphone by the action of CYP3A4 and CYP2D6, respectively (Lalovic et al., 2004). In addition to P450-mediated metabolism, cytosolic aldo-keto reductase also contributes to the reduction of the ketone functionality of oxycodone to afford alpha and beta oxycodol diastereomers (Fig. 1). During the course of this study, we found evidence for extensive formation of noroxycodone and oxymorphone from oxycodone both in vitro and in vivo. Formation of noroxymorphone was observable but it was not formed to the same extent as noroxycodone and oxymorphone. We also observed significant amounts of oxycodone N-oxide formed. We did not examine for the formation of oxycodols.

Previously, the existence of oxycodone N-oxide as a metabolite of oxycodone was postulated (Baldacci et al., 2004; Moore et al., 2003). One report suggested oxycodone N-oxide was formed in small animals but its structure was not confirmed (Ishida, et al., 1979). The report herein presents data for the first time that quantitatively confirms the presence of oxycodone N-oxide as a metabolite of oxycodone both in vitro and in vivo and presents verification of its presence by LCMS-MS. In addition, robust retro-reduction of oxycodone N-oxide to oxycodone was observed. The combined data presented in the report herein appears to, for the first time, complete the description of the Phase I metabolic pathway for oxycodone.

NADPH-dependent formation of oxycodone N-oxide in human liver microsomes was linked to the presence of human FMO3. Human liver microsomes enriched in FMO3 formed more oxycodone N-oxide than microsomes enriched in P450. Heat inactivation of human liver microsomes under conditions that preserved P450 but destroyed FMO3 afforded microsomes that formed less oxycodone N-oxide.

---

**TABLE 11**

Effect of highly purified human FMO3 on oxycodone N-oxide metabolism

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Oxycodone</th>
<th>Oxycodone N-Oxide</th>
<th>Noroxycodone</th>
<th>Oxymorphone</th>
<th>Noroxymorphone</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.001 ± 0.0002</td>
<td>2.4 ± 0.05</td>
<td>0.002 ± 0.0004</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not detected.
The difference between findings reported herein and other previous work (Lalovic, et al., 2004) is likely due to the nature of the metabolite, the thermal instability of FMO3, and the way metabolism studies were conducted. In the absence of NADPH, FMO3 is thermally labile (Cashman, 2005). Typically, about 65%–80% of enzyme activity is lost by treating microsomes or other sources of FMO3 with heat (50–60°C) for as little as 1 to 2 minutes. Traditionally, metabolism studies have been conducted by preheating human liver microsomes at 37°C in the absence of NADPH. Often, incubations have been initiated by addition of NADPH. Under these conditions, most of human FMO3 functional activity is lost. In addition, analytical methods for quantifying tertiary amine N-oxides can be challenging because N-oxide metabolites are polar and not easy to isolate. The analytical methodology used herein was robust and very sensitive.

In rats, oral administration of oxycodone afforded urinary FMO3 metabolite oxycodone N-oxide and the cytochrome P450-mediated metabolites noroxycodone, oxymorphone, and noroxymorphone. Compared with male rats, female rats excreted approximately 13-fold more oxycodone N-oxide. The gender difference may be due to the fact that the amount of FMO3 in young male rats is much lower than that in young female rats (Lattard et al., 2001, 2002). There may also be contributions to gender-specific differences in oxycodone N-oxide reductases (e.g., aldehyde oxidase).

In the presence of liver preparations and hemoproteins, oxycodone N-oxide is very efficiently retro-reduced to oxycodone. Retro-reduction of the N-oxide to oxycodone is a pseudo-futile cycle. In the presence of hepatic preparations, oxycodone is N-oxynated and retro-reduced many times thus appearing to be a futile cycle. However, NADPH is consumed with each cycle. Therefore, this is not a pure futile cycle. Because of its polar nature, oxycodone N-oxide tends not to cross cell membranes and could serve as a reservoir in the tissues where it is formed to provide a sustained source of material to be converted to oxycodone and, in turn, other metabolites.

Data presented herein point to at least three prominent systems responsible for retro-reduction of oxycodone N-oxide. First, aldehyde oxidase makes a contribution to oxycodone N-oxide retro-reduction. Second, similar to studies of other molecules (Kitamura et al., 1999) quinone reductase and hemoglobin makes strong contributions to oxycodone N-oxide retro-reduction. In contrast to other studies (Parte and Kupfer, 2005), however, other hemoproteins such as P450 do not make a significant contribution to oxycodone N-oxide retro-reduction. If FMO3 contributes to retro-reduction, it is likely via a noncatalytic, protein template-type effect previously reported (Cashman et al., 1999). Thus, P450 and FMO3 have a very strong preference for oxidation of oxycodone and not retro-reduction.

Results from in vitro retro-reduction studies described above were consistent with in vivo studies. In rats, after administration of oxycodone N-oxide, the quantitative order of urinary excretion was as follows: oxycodone N-oxide > noroxycodone > noroxymorphone > oxymorphone. Administration of oxycodone N-oxide to rats showed a gender effect: females excreted a greater amount of oxycodone N-oxide than males on the basis of total mass of oxycodone N-oxide, oxycodone, and oxymorphone. Males excreted a higher percentage of noroxycodone and noroxymorphone than females. Again, this may be due to relative functional differences in oxynases and reductases present in male versus female rats.

In humans, after administration of 40 mg PTI-821 (a slow-release formulation of oxycodone) the prevalence (and percent recovery based on total free drug) showed: noroxycodone (27.3%), noroxymorphone (66%), oxycodone (27.1%), oxycodone N-oxide (5.5%), and oxymorphone (0.8%) in the first 12 hours. The percent of the dose of oxycodone excreted in human urine as oxycodone N-oxide is consistent with expectations from in vitro studies. That significant oxycodone

### TABLE 13

<table>
<thead>
<tr>
<th>Description</th>
<th>Oxycodone</th>
<th>Oxycodone N-Oxide</th>
<th>Noroxycodone</th>
<th>Oxymorphone</th>
<th>Noroxymorphone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>0.09 ± 0.006</td>
<td>0.01 ± 0.001</td>
<td>0.02 ± 0.001</td>
<td>0.001 ± 0.0</td>
<td>ND</td>
</tr>
<tr>
<td>+KCN</td>
<td>0.01 ± 0.001</td>
<td>0.004 ± 0.001</td>
<td>0.001 ± 0.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>+Na arsenite+Menadione</td>
<td>0.01 ± 0.001</td>
<td>0.004 ± 0.001</td>
<td>0.001 ± 0.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>+Hemoglobin</td>
<td>0.1 ± 0.002</td>
<td>0.002 ± 0.002</td>
<td>0.005 ± 0.001</td>
<td>0.0004 ± 0.0</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not detected.

### TABLE 14

<table>
<thead>
<tr>
<th>Description</th>
<th>Oxycodone</th>
<th>Oxycodone N-Oxide</th>
<th>Noroxycodone</th>
<th>Oxymorphone</th>
<th>Noroxymorphone</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.004 ± 0.001</td>
<td>14.6 ± 0.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Control</td>
<td>0.01 ± 0.002</td>
<td>15.3 ± 1.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>0.06 ± 0.003</td>
<td>14.3 ± 0.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CYP1A1 + α-Naphthoflavone</td>
<td>0.04 ± 0.02</td>
<td>14.3 ± 0.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>0.02 ± 0.001</td>
<td>1.94 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>CYP2A6 + Tranylcypromine</td>
<td>0.13 ± 0.001</td>
<td>1.9 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>0.06 ± 0.01</td>
<td>1.8 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CYP3A4 + TAO</td>
<td>0.06</td>
<td>1.9 ± 0.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>0.04 ± 0.002</td>
<td>14.2 ± 0.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CYP2D6 + Quinidine</td>
<td>0.02 ± 0.001</td>
<td>13.6 ± 0.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FMO3*</td>
<td>0.002 ± 0.0002</td>
<td>4.4 ± 0.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>FMO3* + MMI</td>
<td>0.002 ± 0.0</td>
<td>4.0 ± 0.2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

MMI, methyl mercapto imidazole, an alternal substrate competitive inhibitor of FMO3; ND, not detected; TAO, troleandomycin.

*Values were expressed as the mean ± S.D. as (nmol/min)/84.5 μg of FMO3, mean of four determinations, pH 7.4.
N-oxide is detected as urinary metabolites in humans suggests that human FMO3 plays a role. As an extension, it may be that gene polymorphisms may play a role in the overall metabolism and distribution of oxycodone (Cashman et al., 2001). Because human FMO3 expression is also developmentally regulated (Koukouritaki et al., 2002), this may also impact the metabolism of oxycodone in the neonate.

In summary, this study showed oxycodone was N-oxidated by human FMO3. Oxycodone N-oxide was shown to be retro-reduced to oxycodone by at least three enzymatic systems including AO, quinone reductase and hemoglobin. Oxycodone N-oxide possesses some novel properties in being polar yet sufficiently lipophilic to remain in the endoplasmic reticulum to be converted to oxycodone. Thus, oxycodone N-oxide may serve in a depot effect for metabolic conversion to oxycodone.

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Authorship Contributions

Participated in research design: Cashman, de Kater, Schoenhard.
Conducted experiments: Cashman, Gohdes.
Contributed new reagents or analytical tools: Gohdes.
Performed data analysis: Gohdes, de Kater, Schoenhard.
Wrote or contributed to the writing of the manuscript: Cashman, de Kater, Schoenhard.

References


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