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Characterization of oxycodone *in vitro* metabolism by human cytochromes P450 and UDP-glucuronosyltransferases

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ABSTRACT

The hepatic metabolism of oxycodone by cytochromes P450 (CYP) and the UDP-glucuronosyltransferases (UGT), the main metabolic enzymes of phase I and phase II, respectively, was assessed *in vitro*. The *N*-demethylation by CYP3A4/5 and the *O*-demethylation by CYP2D6 in human liver microsomes (HLM) followed Michaelis-Menten kinetics, with intrinsic clearances of 1.46 μL/min/mg and 0.35 μL/min/mg, respectively. Although noroxycodone and oxymorphone mainly contribute to the elimination of oxycodone, the simulated total *in vivo* clearance using *in vitro* phase I metabolism was underestimated. For the first time, metabolism of oxycodone by UGT was deeply investigated using HLM, recombinant enzymes and selective inhibitors. Oxycodone-glucuronide was mainly produced by UGT2B7 (K_m = 762 ± 153 μM, V_{max} = 344 ± 20 peak area/min/mg) and to a lesser extent by UGT2B4 (K_m = 2454 ± 497 μM, V_{max} = 201 ± 19 peak area/min/mg). Finally, the kinetics of the drug–drug interactions were assessed using two CYP and UGT cocktail approaches. Incubations of HLM with phase I and phase II drug probes showed that oxycodone mainly decreased the *in vitro* activities of CYP2D6, CYP3A4/5, UGT1A3, UGT1A6 and UGT2B subfamily with an important impact on UGT2B7.

1. Introduction

Oxycodone (4,5-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one) is a semisynthetic opioid that acts as an agonist of the μ and κ receptors in the central nervous system [1]. This drug is used to treat moderate to severe pain [2]. Oxycodone is extensively metabolized and bioactivated by hepatic cytochromes P450 (CYP), and less than 10% of the dose is excreted in urine in its unchanged form [3]. Generally, phenanthrene opioids undergo conjugative metabolism by uridine diphosphate (UDP) glucuronosyltransferases (UGT) in the liver and are excreted in urine [4,5]. A small amount of oxycodone was shown to be excreted as conjugated form by analyzing human urine before and after treatment with β-glucuronidase [6,7]. Although CYP-dependent metabolism of oxycodone is well established, the involvement of UGT in biotransformation is currently unclear. The main phase I metabolite, noroxycodone (Fig. 1), is produced by *N*-demethylation at position 17 by CYP3A4/5, whereas the *O*-demethylation of oxycodone into oxymorphone is catalyzed by CYP2D6 [8,9]. The active *O*-demethylated metabolite is 14 times more potent than oxycodone and its affinity for the μ-opioid receptor is 3- to 5-fold higher than morphine [10]. In contrast to morphinan derivatives, oxycodone possesses a methyl group at the 3-position and a keto group at the 6-position, making the hydroxyl group attached to carbon 14 the only position available for *O*-glucuronidation. To date, no data about the *in vitro* glucuronidation of oxycodone by human liver microsomes (HLM) or recombinant UGTs are available. The involvement of different metabolic pathways in the biotransformation of oxycodone could potentially generate clinically meaningful changes in the drug pharmacokinetics (PK), leading to adverse effects or reduced analgesia [11]. Currently, more comprehensive estimations of the magnitude of pharmacokinetic changes are made possible by using a physiologically based pharmacokinetic (PBPK) model, in which drug, body and trial components interact with each other in the same dynamic *in silico* model [11]. In the so-called *bottom-up* strategy, predictions

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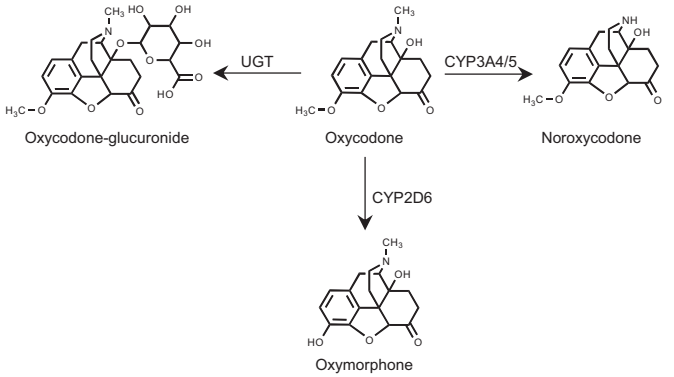

Fig. 1. Metabolic pathways of oxycodone.

are obtained by integrating the drug molecular descriptors and *in vitro* PK parameters in the model [12].

The aim of this study was to characterize the *in vitro* metabolism of oxycodone by the two major drug-metabolizing enzymes pathways, phase I (CYPs) and phase II (UGTs), using ultra-high performance liquid chromatography–mass spectrometry (UHPLC–MS). The UGT isoforms responsible for oxycodone glucuronidation were identified using recombinant UGTs. Inhibition of the formation of phase I and phase II metabolites using CYP-selective and UGT-selective inhibitors was also studied to confirm the isozymes involved in oxycodone metabolism. Finally, the inhibitory effect of oxycodone on the microsomal CYP and UGT isoforms was assessed using two distinct cocktail approaches to evaluate the potential risk of drug–drug interactions involving this compound.

2. Materials and methods

2.1. Chemicals

Oxymorphone, oxymorphone-d3, noroxycodone, noroxycodone-d3, buproprion hydrochloride, dextromethorphan hydrobromide, phenacetin, flurbiprofen, coumarin, etoposide, chenodeoxycholic acid, isoferulic acid, azidothymidine, trifluoperazine dihydrochloride, serotonin hydrochloride, testosterone, fluconazole, naloxone, uridine diphosphate-glucuronic acid (UDPGA), fluoxetine hydrochloride, quinidine anhydrous, ketoconazole, potassium hydroxide, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt (HEPES), dimethylsulfoxide, and ammonium hydroxide were provided by Sigma-Aldrich (Steinheim, Germany). Oxycodone hydrochloride monohydrate, codeine hydrochloride, *d*,l-4-hydroxy-3-methoxymethamphetamine hydrochloride, venlafaxine hydrochloride and midazolam were obtained from Lipomed AG (Arlesheim, Switzerland). Levemedetomidine and chlorzoxazone were supplied by Toronto Research Chemicals Inc. (Toronto, Canada). (*S*)-mephenytoin was obtained from Enzo Life Sciences (Lausen, Switzerland). Formic acid was purchased from Merck (Darmstadt, Germany), whereas leucine-enkephalin was obtained from Bachem (Bubendorf, Switzerland). Reduced β-nicotinamide adenine dinucleotide 2′-phosphate tetrasodium salt (NADPH) was provided by Applichem (Darmstadt, Germany). Acetonitrile (ACN) and methanol of ULC–MS grade were obtained from Biosolve (Valkenswaard, Netherlands). Pooled human liver microsomes (HLM) from 20 donors, UGT Supersomes from baculovirus-infected insect cells and reaction mixture-Solution B (250 mM TRIS*HCl, 40 mM $MgCl_2$, 0.125 mg/mL alamethicin and 1.25% (v/v) methanol in water) were purchased from Corning (Amsterdam, The Netherlands). Ultra-pure water was supplied by a Milli-Q purification unit from Millipore (Belfort, NY, USA).

2.2. Solutions

Stock solutions were prepared at different concentrations in water (oxycodone, serotonin, trifluoperazine, azidothymidine, naloxone, fluconazole, NADPH, UDPGA), in methanol (oxymorphone, oxymorphone-d3, noroxycodone, noroxycodone-d3, codeine, *d*,l-4-hydroxy-3-methoxymethamphetamine, chenodeoxycholic acid, levomedetomidine, venlafaxine, fluoxetine, ketoconazole, quinidine, phenacetin, coumarin, bupropion, flurbiprofen, (*S*)-mephenytoin, dextromethorphan, chlorzoxazone and midazolam) or in dimethylsulfoxide (etoposide, testosterone, isoferulic acid). Intermediate concentrations and working solutions were prepared according to the reports of Spaggiari or Gradinaru for phase I and phase II protocols, respectively [13,14].

2.3. Incubation procedures for CYP450- and UGT-dependent metabolism

For experiments regarding the formation of oxymorphone and noroxycodone, HLM (0.5 mg protein/mL) were incubated in a reaction medium containing oxycodone, NADPH and HEPES (pH 7.4, 50 mM) buffer solution. The buffer was prepared by dissolving the required amount of HEPES in water and the pH was adjusted to 7.4 with potassium hydroxide. The final incubation volume was 100 μL and the final concentration of the organic solvent (methanol) was less than 0.5% (v/v). After a 3 min preincubation at 37 °C, the reaction was initiated by adding 2 mM NADPH. The incubation proceeded for 20 min at 37 °C under agitation (400 rpm). The enzymatic reaction was stopped by adding 100 μL of ice-cold ACN containing oxymorphone-d3 and noroxycodone-d3 (final concentration 500 ng/mL). The precipitated proteins were removed by centrifugation (5 min at 10,000 rpm) and an aliquot of the resulting supernatant was transferred to a vial for UHPLC–MS analysis.

Regarding the glucuronidation of oxycodone, HLM or recombinant UGTs (0.5 mg protein/mL) were mixed with the incubation medium containing TRIS*HCl buffer (50 mM, pH 7.5), alamethicin (25 μg/mL) and $MgCl_2$ (8 mM), with the final concentrations given in brackets. After 10 min on ice, oxycodone was added and preincubated for 10 min at 37 °C under agitation (400 rpm). The reaction was then initiated by adding the UDPGA cofactor (4 mM) to a total volume of 100 μL. The final concentration of the organic solvent was less than 0.5% (v/v). The incubation proceeded for 80 min at 37 °C under agitation (400 rpm) and was stopped by adding 100 μL of ice-cold ACN to the reaction medium. The precipitated proteins were removed by centrifugation (10,000 rpm for 10 min) and the supernatant was transferred to a vial for UHPLC–MS analysis.

Each experiment was performed in duplicate or triplicate, and blank reactions were performed in the absence of NADPH or UDPGA. The formation of oxymorphone, noroxycodone and oxycodone-glucuronide was linear with respect to the selected protein concentration and incubation times.

2.4. UGT mapping for oxycodone metabolism

In vitro screening of ten UGT Supersomes (1A1, 1A3, 1A4, 1A6, 1A9, 2B4, 2B7, 2B10, 2B15, and 2B17) that were individually incubated with oxycodone was performed to identify the isoforms involved in its glucuronidation. Incubations with oxycodone (0.5 mM) were performed according to the glucuronidation procedure described in Section 2.3.