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Research Article

Evaluation of *In-vitro* Metabolism of Oxycodone in Pediatric Population -

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ABSTRACT

Oxycodone is a semi-synthetic opioid analgesic used in the management of postoperative pain and chronic cancer pain. It is extensively metabolized by the liver to noroxycodone, oxymorphone and noroxymorphone via N-demethylation and/ or O-demethylation reactions. In adults, CYP3A4 and CYP2D6 are the major CYP-isoforms responsible for the formation of noroxycodone, oxymorphone and noroxymorphone. The present study was undertaken to study the oxidative and conjugative metabolism of oxycodone in a pediatric population using both liver microsomes and hepatocytes. Liver microsomes from various pediatric populations catalyzed the formation of noroxycodone as the major in vitro metabolite of oxycodone followed by oxymorphone and noroxymorphone. The K_m and V_{max} values for noroxycodone formation in the liver microsomes tested from individual pediatric donors ranged between 19.9 ± 8.22 – 395.6 ± 114.5 µM and 104.2 ± 10.32 – 2529 ± 203.9 pmol/ min/ mg, respectively. The corresponding values for the pooled adult human liver microsomes were 133.1 ± 33.84 µM and 1821 ± 160.4 pmol/min/mg, respectively, and within the pediatric range. The Km and V_{max} values for oxymorphone formation in the liver microsomes tested from individual pediatric donors ranged between 3.83 ± 1.54 to 230.2 µM and 18.6 ± 2.96 to 132.5 ± 26.0 pmol/ min/ mg, respectively, respectively. The corresponding values for the pooled adult human liver microsomes were 107.0 ± 70.8 µM and 83.7 ± 28.4 pmol/ min/ mg, respectively, and within the pediatric range. These results indicate that liver plays a major role in the oxidative metabolism of oxycodone in pediatrics. CYP3A-isoforms appear to play a major role in the metabolic clearance of oxycodone in all age groups. A considerable variation was seen in *in vitro* intrinsic clearance (V_{max}/ K_m) values in pediatrics which, may be due to the expression of elevated levels of drug metabolizing enzymes responsible for oxycodone metabolism. A significant inter-individual variability in the formation of relative amounts of oxycodone metabolites was observed in microsomes from different age groups of pediatrics. However, no pediatric age group-specific qualitative differences in oxycodone metabolites were observed. In the clinic, the dose of oxycodone must be personalized individually depending on their age.

Keywords: Pediatrics; drug metabolism; oxycodone; noroxymorphone; microsomes; hepatocytes

ABBREVIATIONS

CYP: Cytochrome P450; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; PB: Phosphate Buffer; KTZ: Ketoconazole; QUIN: Quinidine; UDPGA: Uridine 5'-Diphosphoglucuronic Acid

INTRODUCTION

Oxycodone (4,5 α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one; 14-dihydrohydroxycodeinone) is a semi-synthetic opioid analgesic used in the management of postoperative pain and chronic cancer pain [1-4]. It has also been suggested for the management of nonmalignant chronic pain [5,6]. It is extensively metabolized through a series of separate metabolic pathways that includes: N-demethylation, O-demethylation, N-oxidation, 6-keto reduction and conjugation (Figure 1). Noroxycodone and oxymorphone are the primary metabolic products of N-demethylation and O-demethylation reactions, respectively [7-10]. Oxymorphone is a minor metabolite of oxycodone but has 3 to 5- times higher mu-opioid receptor affinity than morphine [11]. Oral oxymorphone is nearly 10-fold more potent than oral morphine based on dose more active metabolite of oxycodone [12]. Noroxymorphone was shown to be a secondary oxidative metabolite of oxycodone [13]. In humans, multiple cytochrome P450s (CYPs) appear to be involved in the oxidative metabolism of oxycodone, with CYP3A4 and CYP2D6 being the primary enzymes responsible in the formation of noroxycodone and oxymorphone, respectively. The reductive and conjugative pathways of oxycodone are poorly understood. However, cytosolic ketoreductases may be involved in the formation of 6α- and 6β-oxycodols. Although oxycodone has been used for many years in pain management, metabolism of oxycodone in pediatric population is not well studied.

The present study was undertaken to evaluate the *in vitro* metabolism of oxycodone in pediatric populations using both liver microsomes and hepatocytes from representative age groups (Tables 1-3), and to compare the metabolic profile and kinetics with those from adults.

MATERIALS AND METHODS

Materials

Oxycodone hydrochloride (Lot # 8862SPL006), noroxycodone (Lot # 33387-84), oxymorphone (Lot # 34698-8) and noroxymorphone (Lot # V14735) were obtained from the Pharmacy, Purdue Pharma LP. BCA Protein Assay Kit was purchased from Pierce Chemical (Rockford, IL). NADPH, UDPGA, alamethicin, saccharolactone and testosterone were purchased from Sigma Chemical Co. (St. Louis, MO). Bufuralol, 1'-hydroxybufuralol and 6β-hydroxytestosterone were obtained from BD Gentest Corp. (Woburn, MA). All chemicals and solvents used were of the analytical reagent grade of highest purity.

Preparation and Characterization of Liver Microsomes and Hepatocytes

Pooled adult human liver microsomes were prepared by

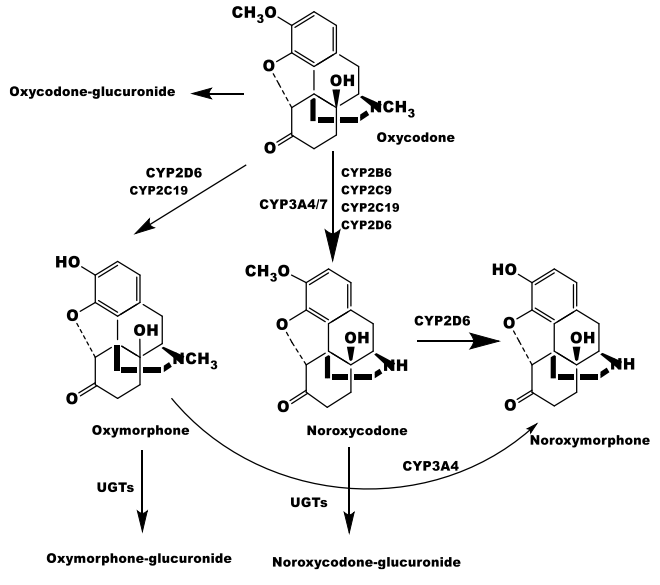

Figure 1: Schematic Representation of Oxycodone and its Metabolites.

Table 1: Age Group Categories.

Age Group	Age	No. of Microsomal Samples	No. of Hepatocyte Samples
Neonate	Birth to 1 mo.	-	1
Infant	1 mo. to 2 yr	4	1
Children	2 to 12 yr	7	1
Adolescent	12 yr to < 16 yr	1	0
Adult	> 18 yr	1	1

Table 2: Donor Information for Individual Human Liver Microsome Samples.

Pediatric Liver Samples	Gender	Age	Race
H0039	Male	8 yrs.	Cauc*.
H0053	Male	8 mos.	Afr. Am.
H0054	Female	12 yrs.	Afr. Am.
H0055	Male	8 yrs.	Hisp.
H0056	Male	8 yrs.	Afr. Am.
H0057	Female	2 yrs.	Cauc.
H0058	Female	6 yrs.	Cauc.
H0059	Male	9 yrs.	Cauc.
H0215	Male	6 yrs.	Cauc.
H0238	Male	3 mos.	Afr-Am.
H0268	Male	4 mos.	Cauc.
H0282	Male	2 mos.	Hisp.

*Cauc: Caucasian; Afr Am: African American; Hisp: Hispanic

Table 3: Donor Demographics and Medical Histories of Pediatric and Adult Human Cryopreserved Hepatocytes.

Lot #	Age	Sex	Race	Cause of Death	Medical History
RQO	6-day	Female	C*	Anoxia	None
079	9-months	Male	C	Head Trauma	Jaundice
130	2 years	Female	C	Anoxia	None
122	42 years	Male	H	Intracranial hemorrhage	Hypertension, Epilepsy

*C: Caucasian; H: Hispanic

differential centrifugation of tissue homogenates of human liver tissue samples according the described method [14]. Pediatric human liver microsomes were purchased from XenoTech, LLC (Kansas City, KS). All the pediatric human liver microsomes were characterized for total cytochrome P450 content, cytochrome b5, NADPH-cytochrome c reductase, 7-ethoxyresorufin O-dealkylase (CYP1A2), coumarin 7-hydroxylase (CYP2A6), S-mephenytoin N-demethylase (CYP2B6), paclitaxel 6α-hydroxylase (CYP2C8), diclofenac 4'-hydroxylase (CYP2C9), *S*-mephenytoin 4'-hydroxylase (CYP2C19), dextromethorphan O-demethylase (CYP2D6), chlorzoxazone 6-hydroxylase (CYP2E1), testosterone 6β-hydroxylase (CYP3A4), lauric acid 12-hydroxylase (CYP4A9/ 11) and CYP2D6 genotype analysis (data not included). Microsomes were stored at -80°C until use. Total microsomal protein concentration was measured using a Protein Assay Kit with bovine serum albumin as the reference standard [15]. The total cytochrome P450 content was measured as spectrophotometrically by the described method [16].

The cryopreserved hepatocytes were obtained from In Vitro Technologies (Baltimore, MD). All cryopreserved hepatocytes were also characterized for coumarin 7-hydroxylase, dextromethorphan O-demethylase, 7-ethoxycoumarin O-deethylase, 7-hydrocoumarin glucuronidase, 7-hydroxycoumarin sulfate, S-mephenytoin 4'-hydroxylase, testosterone 6β-hydroxylase, tolbutamide 4-hydroxylase, phenacetin O-deethylase and chlorzoxazone 6-hydroxylase (data not included).

Microsomal Incubations with NADPH

All pediatric human liver microsomes (1 mg/ mL, final concentration) were incubated with oxycodone (5, 10, 25, 50, 100, 200, 400 and 800 μM, final concentration) in a reaction volume of 0.2 mL. The reaction mixture also contained 50 mM potassium phosphate buffer, pH 7.4, 5 mM $MgCl_2$ and 1 mM EDTA. The reactions were initiated by the addition of NADPH (1 mM, final concentration) and the samples were incubated at 37˚C in a water bath shaker for 0, 15, 30 and 60 minutes. Incubations were also carried out using pooled adult human liver microsomes. All microsomal incubations were carried out in 15 mL screw-top glass tubes in triplicate. Incubations with no liver microsomal protein and no NADPH for 0 and 60 minutes were run as negative controls.

The rates of formation of noroxycodone and oxymorphone were determined at eight different concentrations of oxycodone ranging from 0-800 μM. The data on the rates of formation of noroxycodone and oxymorphone were fitted to following equation.

$$v = \frac{Vmax \cdot S}{Km + S}$$

Where, v represents the rate of formation of metabolite, K_m the Michaelis-Menten constant, V_{max} the maximum rate of formation of a metabolite, and S the concentration of substrate

Microsomal Incubations with UDPGA

All pediatric human liver microsomes (1 mg/ mL, final concentration) were incubated separately with oxycodone (750 μM, final concentration), oxymorphone (750 μM, final concentration) and noroxycodone (750 μM, final concentration) in a reaction volume of 0.25 mL. The reaction mixture also contained 50 mM potassium phosphate buffer, pH 7.4, 5 mM $MgCl_2$, 1 mM EDTA, 8.5 mM saccharolactone and 25 μg of alamethicin in a reaction volume of 0.25 mL. The reactions were initiated by the addition of UDPGA (2 mM, final concentration). The samples were incubated at 37˚C water bath shaker for 0 and 60 minutes. Incubations were also carried out using pooled adult human liver microsomes. All the incubations were carried out in screw-top glass tubes in triplicate. Incubations with no microsomal protein and no UDPGA were carried out for 0 and 60 minutes and served as negative controls.

Incubation with Hepatocytes

In order to investigate the formation of both oxidative and conjugative metabolites of oxycodone, oxymorphone and noroxycodone, the cryopreserved hepatocytes (1 x 10^6 cells/ mL) were incubated individually with oxycodone, oxymorphone or noroxycodone (100 μM, final concentration) in a total volume of 0.25 mL of incubation media. The incubations were carried out in duplicate at 37˚C in a 5% CO_2 incubator for 0, 60 and 120 minutes. Incubations were also carried out using cryopreserved adult human hepatocytes. Incubations with no hepatocytes in the presence of oxycodone, oxymorphone and noroxycodone were carried out for 0, 60 and 120 minutes and served as negative controls.

Sample Processing

For the analysis of oxycodone and its oxidative metabolites, the incubations were terminated by the addition of 4 mL of dichloromethane. The samples were mixed vigorously and centrifuged

at 3000 rpm for 10 minutes at 4˚C. The dichloromethane layer was carefully removed, transferred into another tube, and evaporated to dryness under a stream of nitrogen at 40˚C using a TurboVap LV evaporator (Zymark Corp., Hopkinton, MA). The dried samples were reconstituted in 100 µL mobile phase and transferred to 96-well plates. An aliquot of 50 µL was analyzed using reverse-phase HPLC according to the method described below.

For the analysis of conjugated metabolites of oxycodone, oxymorphone and noroxycodone, the incubations were terminated by the addition of equal volume of ice-cold methanol. The samples were centrifuged at 5000 rpm for 10 minutes at 4˚C to remove any protein precipitate. The clear supernatant was removed and transferred to 96-well plates, and an aliquot (50 µL) was analyzed on reverse-phase HPLC.

Metabolite Analysis

HPLC Analysis: Oxycodone and its metabolites in liver microsomes and hepatocytes were analyzed on reverse phase HPLC and LC-MS. The HPLC analysis was performed on a Waters Alliance 2790 HPLC system connected to a Waters dual wavelength absorbance detector (model 2487) (Waters Corp., Milford, MA). The separation of the metabolites was performed using a C_{18} column (X-Terra, RP, 3.5 µm, 3.0 x 150 mm; Waters Corp., MA). The mobile phase A and B consisted of acetonitrile and 0.16% ammonium carbonate, respectively with a linear gradient (initial condition up to 2 min,100 percent mobile phase B; 40 percent mobile phase B up to 25 min; 20 percent mobile phase B at 26 min and 100 percent mobile phase B up to 30 min). The flow rate was maintained at 0.7 mL/ min, the eluates were monitored at 280 nm, with a column temperature of 40˚C. Oxycodone, noroxycodone, oxymorphone and noroxymorphone were eluted at approximately at 15.4, 10.2, 12.4 and 5.5 min, respectively. The data was acquired using Millennium [32] software version 3.2 (Waters Corp., Milford, MA). The amounts of noroxycodone, oxymorphone and noroxymorphone formed in the *in vitro* samples were measured using respective calibration curves (range between 1 and 5000 pmol). Unless otherwise stated, because of non-availability of authentic glucuronide reference standards, the presence of glucuronides of oxycodone, oxymorphone and noroxycodone was confirmed using LC-MS analysis. Data were analyzed using Microsoft Excel 97, Sigma Plot and/ or GraphPad (Prism 3.0).

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis: Glucuronides of oxycodone, oxymorphone and noroxycodone were analyzed on LC-MS using the following conditions: Column, Phenomenex 5 m Phenol-hexyl (100 x 4.6 mm); Mobile phase, 10 mM ammonium acetate; Gradient, 0 (5% mobile phase B) to 10 min (27% mobile phase B) to 10.01 min (100% mobile phase B) to 12 min (100% mobile phase B) to 12.01 min (5% mobile phase B) to 16 min (5% mobile phase B); flow rate, 1 mL/ min. The mass spectrometer was operated in the atmospheric pressure ionization electrospray mode with positive polarity.

RESULTS

Oxycodone Metabolism in Pediatric Liver Microsomes and the Determination of Michaelis-Menten Constants

As per the FDA Pediatric Guidelines, the pediatric human liver microsomes and hepatocytes samples used in this study are classified into several age groups (Table 1). The summary of all donor history of liver microsomes is presented in Table 2. All pediatric human liver microsomes tested catalyzed the formation of noroxycodone as the major metabolite followed by oxymorphone and noroxymorphone, in the presence of NADPH (Figures 2 and 3). The conversion of oxycodone to noroxycodone and oxymorphone in pediatric microsomes was proportional to the incubation time, which is similar to the adult human liver microsomes. As shown in Figures 4 and 5, a significant inter-individual variability in the relative amounts of noroxycodone and oxymorphone formation was observed in pediatrics.

A series of incubations were carried out to ascertain the effects of various concentrations of oxycodone on the formation of its metabolites, from which Michaelis-Menten kinetic constants (K_m and V_{max}) were determined. Figures 6-8 shows representative saturation

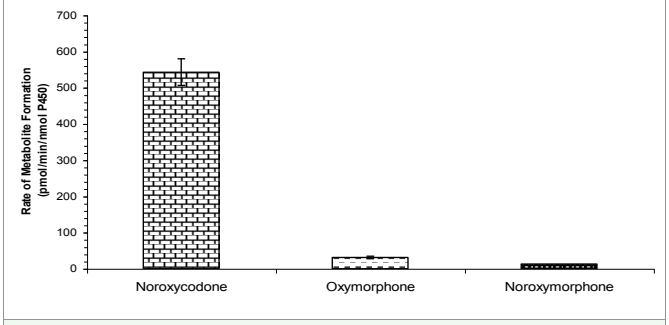

Figure 2: A Representative Profile on the Formation of Oxycodone Metabolites from Oxycodone (200 µm) by Pediatric Liver Microsomes (Sample L, 2 Month, Male, Hispanic).

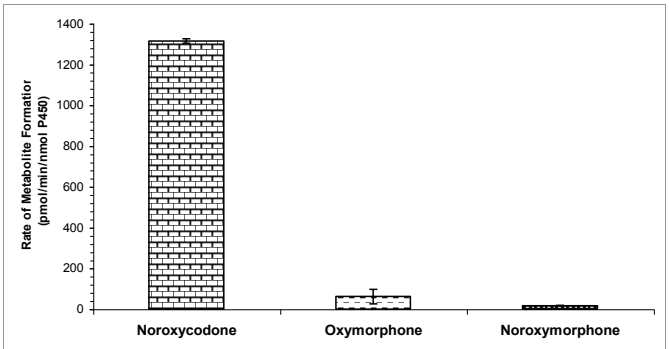

Figure 3: A Representative Profile on the Formation of Oxycodone Metabolites from Oxycodone (200 µm) by Adult Human Liver Microsomes (Sample M, Pooled Adult, Average Age 45.5 Yrs).

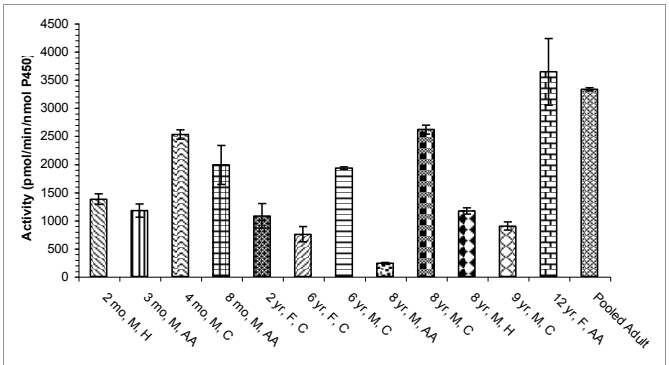

Figure 4: Inter-Individual Variation in the Formation of Noroxycodone from Oxycodone in Pediatric Vs. Adult Liver Microsomes.
M: Male; F: Female; C: Caucasian; AA: African American; H: Hispanic.

profiles of noroxycodone formation in two pediatric microsomal preparations, which illustrates different kinetic behavior between two liver samples. The K_m and V_{max} values for noroxycodone formation in pediatric liver microsomes ranged from 19.8 ± 8.22 to 395.6 ± 114.5

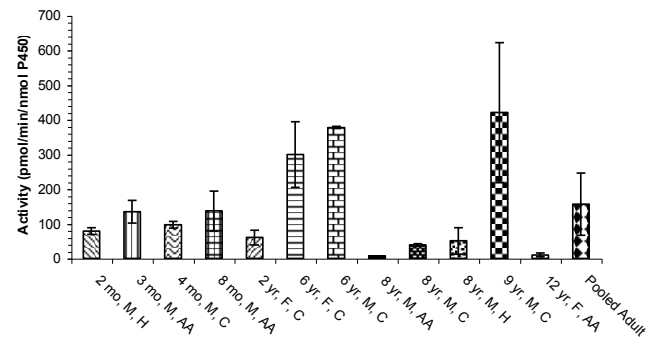

Figure 5: Inter-Individual Variation in the Formation of Oxymoprhone from Oxycodone in Pediatric Vs. Adult Liver Microsomes.
M: Male; F: Female; C: Caucasian; AA: African American; H: Hispanic.

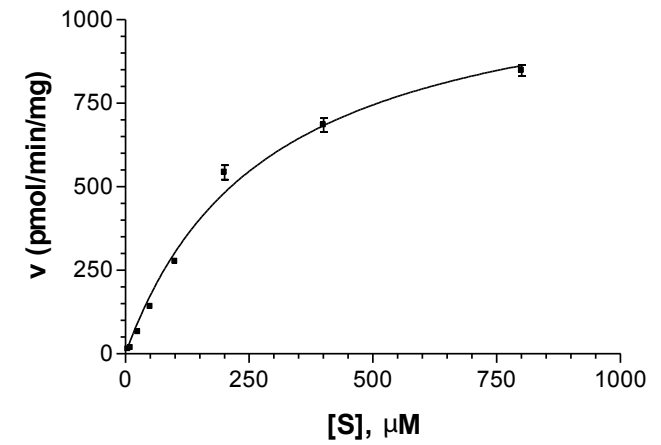

Figure 6: Michaelis-Menten Plot for the Determination of Km and Vmax for Noroxycodone from Oxycodone in Human Pediatric Liver Microsomes (Sample L, 2 Mo, Male, and Hispanic).

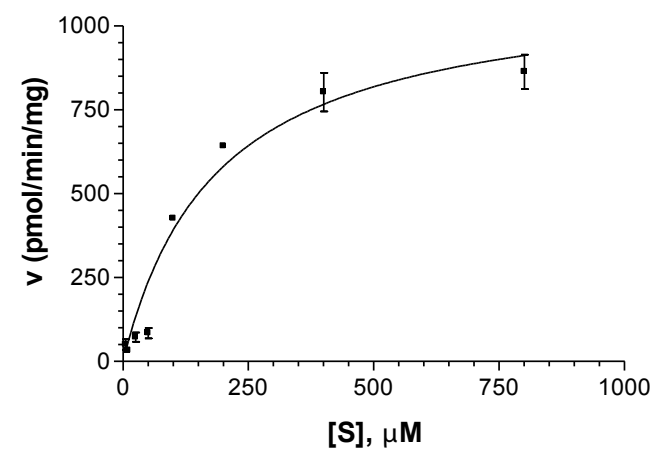

Figure 7: Michaelis-Menten Plot for the Determination of Km and Vmax for Noroxycodone in A Pediatric Human Liver Microsomes (Sample I, 6 Yr., Male, Caucasian)

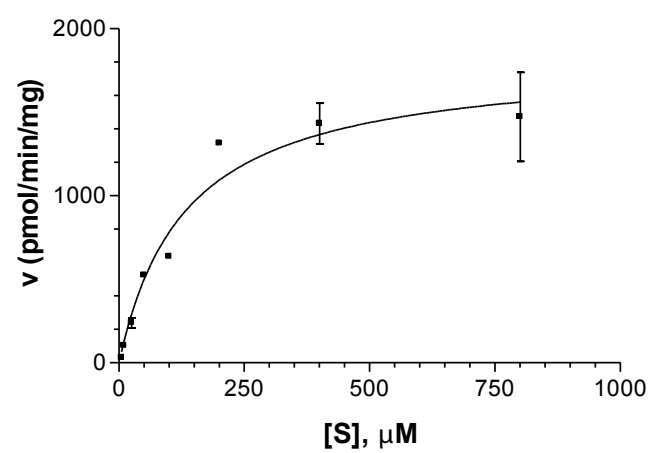

Figure 8: A Michaelis-Menten Plot for the Determination of Km and Vmax for Noroxycodone from Oxycodone in Pooled Adult Human Liver Microsomes.

µM and 104.2 ± 10.3 to 2529 ± 203.9 pmol/ min/ mg, respectively (Table 4). These results suggest a nearly 20- and 24-fold variation in Km and Vmax values respectively for noroxycodone formation in pediatric populations. An approximately 10-fold variation was observed in K_m values for noroxycodone formation between both infants and children populations, and a nearly 5- and 23-fold variation in the V_{max} values was observed for noroxycodone formation between infant and children groups, respectively. The K_m and V_{max} values for noroxycodone formation in adult liver microsomes are 133.1 ± 34.9 µM and 1821 160.4 pmol/ min/ mg, these values are well within the range compared to other pediatric liver microsomes. The enzyme responsible for norhydrocodone formation in adults is shown to be CYP3A4 and it appears to be a "low-affinity" and "high-capacity" enzyme.

The Km and Vmax values for oxymorphone formation in pediatric liver microsomes range from 3.83 ± 1.54 to 230.2 µM and 18.6 ± 2.96 to 132.5 ± 26.0 pmol/ min/ mg, respectively (Table 5). These results suggest a nearly 60- and 7-fold variation in Km and V_{max} values respectively for oxymorphone formation. The K_m and V_{max} values for oxymorphone in adult human liver microsomes are 107.0 ± 70.8 and 83.7 ± 28.4 pmol/ min/ mg, respectively and these values are well within the range of Km and Vmax values in pediatric populations. The major CYP enzyme responsible for oxymorphone formation in humans is CYP2D6 and appears to be a "high-affinity" and "low-capacity" enzyme.

The *in vitro* intrinsic clearance (V_{max}/ K_m) values were determined for noroxycodone and oxymorphone formation in oxycodone metabolism and are presented in Tables 5 and 6, respectively. An approximately 25- and 71-fold variation in the in vitro clearance values for both noroxycodone and oxymorphone formation, respectively, was observed. The intrinsic clearance values for noroxycodone were slightly higher than the oxymorphone intrinsic clearance values, suggesting that noroxycodone is the major metabolite of oxycodone and the CYP3A4/ 7 derived pathway appears to be the major route of elimination in vivo. Alternatively, the in vitro intrinsic clearance value for noroxycodone is nearly one order of magnitude higher than the intrinsic values for oxymorphone formation in pooled adult liver microsomes and appears to be the major route of elimination in vivo. A marked variation in oxycodone pharmacokinetics in infants [17] and the association between CYP2D6 genetic variability [18] have been reported.

Table 4: Summary of *In vitro* Kinetic Constant (K_m, V_{max} and V_{max}/K_m) values for Noroxycodone formation from Oxycodone in different Human Pediatric and Adult Liver Microsomal Samples.

Sample ID	Sample Number	Donor History	K_m, (μM)	V_{max} (pmol/ min/ mg)	V_{max}/K_m μl/ min/ mg)
A	H0039	8 yr, M, C	260.8 ± 56.4	1905 ± 182.6	7.32
B	H0054	12 yr, F, AA	66.59 ± 36.62	2421 ± 372	36.35
C	H0055	8 yr, M, H	82.68 ± 54.25	1301 ± 351.9	15.73
D	H0056	8 yr, M, AA	48.18 ± 14.31	201.4 ± 20.83	4.18
E	H0057	2 yr, F, C	193 ± 86.68	559.5 ± 119.5	2.89
F	H0058	6 yr, F, C	30.6 ± 11.06	104.2 ± 10.32	3.40
G	H0059	9 yr, M, C	19.88 ± 8.22	107.9 ± 10.08	5.42
H	H0053	8 mo, M, AA	40.35 ± 20.53	1274 ± 165	31.57
I	H0215	6 yr, M, C	187.3 ± 36.47	1126 ± 81.72	6.01
J	H0238	3 mo, M, AA	395.6 ± 114.5	505.7 ± 68.88	1.28
K	H0268	4 mo, M, C	388.5 ± 67.23	2529 ± 203.9	6.50
L	H0282	2 mo, M, H	285.9 ± 29.75	1171 ± 52.71	4.09
M	Pooled Adult	45.5 yr. (average)	133.1 ± 34.97	1821 ± 160.4	13.68

M: Male; F: Female; C: Caucasian; AA: African American; H: Hispanic

Table 5: Summary of *In vitro* Kinetic Constant (K_m, V_{max} and V_{max}/K_m) values for Oxymorphone formation from Oxycodone in different Human Pediatric and Adult Liver Microsomal Samples.

Sample ID	Sample Number	Donor History	K_m, (μM)	V_{max} (pmol/ min/ mg)	V_{max}/K_m μl/ min/ mg)
A	H0039	8 yr, M, C	15	30.16	2.01
B	H0054	12 yr, F, AA	7.5	49.31	6.57
C	H0055	8 yr, M, H	39.65 ± 35.76	82.02 ± 25.58	2.06
D	H0056	8 yr, M, AA	7.5	51.22	6.82
E	H0057	2 yr, F, C	15	95.68	6.37
F	H0058	6 yr, F, C	15	126.7	8.44
G	H0059	9 yr, M, C	7.5	128.1	17.08
H	H0053	8 mo, M, AA	4.15 ± 1.95	97.69 ± 7.88	23.49
I	H0215	6 yr, M, C	47.05 ± 25.76	132.5 ± 26.00	2.81
J	H0238	3 mo, M, AA	13.95 ± 8.35	18.61 ± 2.960	1.33
K	H0268	4 mo, M, C	336.3 ± 60.37	99.62 ± 7.91	0.30
L	H0282	2 mo, M, H	3.35 ± 1.453	38.90 ± 2.60	11.59
M	Pooled Adult	45.5 yr. (average)	25.23 ± 8.68	34.62 ± 3.43	1.37

M: Male; F: Female; C: Caucasian; AA: African American; H: Hispanic

The incubations of oxycodone in the absence of various pediatric liver microsomes and NADPH (negative control incubations), resulted in no formation of noroxycodone, oxymorphone or noroxymorphone. These results suggest that the formation of noroxycodone, noroxymorphone and oxymorphone from oxycodone is NADPH-dependent and these are the oxidative metabolites of oxycodone that are catalyzed by the oxidative enzymes in the liver microsomes.

All of the pediatric liver samples were subjected to CYP2D6 genotyping. The results showed that no poor metabolizers of CYP2D6 were observed which is consistent with the determination of CYP2D6 marker enzymatic activity as well as oxycodone O-demethylase activity (oxymorphone formation) in these microsomal samples. Glucuronidation of Oxycodone and its Metabolites

In order to determine the extent of glucuronidation of oxycodone and its metabolites (noroxycodone and oxymorphone), liver microsomes isolated from various pediatric populations were incubated separately with oxycodone, noroxycodone and oxymorphone in the presence of UDPGA. A noticeable inter-individual difference in the relative amounts of glucuronides was observed in pediatric liver microsomes. The qualitative amounts of oxycodone, noroxycodone and oxymorphone glucuronides in pediatric samples were 216.2 ± 3.79 - 1168.9 ± 102.6, 230 ± 10.9 - 1281.5 ± 438.6, and 438.1 ± 41.7 – 2387.5 ± 260.8 pmol/ min/ mg. The relative amounts of oxymorphone glucuronide were approximately 1.5 - 2- times higher than the oxycodone and noroxycodone glucuronides. These results suggest a nearly 5-6-fold variation in the relative amounts of each of the glucuronides being formed in pediatric liver microsomes. The qualitative amounts of oxycodone, noroxycodone and oxymorphone glucuronides in adult human liver microsomes is well within the range of these glucuronides formed by pediatric liver microsomes tested. The fold difference in relative amounts these glucuronides in pediatric liver microsomes may in part related to the elevated levels of expression of enzymes responsible for the formation of respective glucuronides. Overall, these results suggest that although the enzymes (UGTs) responsible for the formation of glucuronides of oxycodone, noroxymorphone and oxymorphone are not known at present, these enzymes appear to be equally expressed in pediatrics population.

Oxycodone, Noroxycodone and Oxymorphone Metabolism using Pediatric Human Hepatocytes

In order to investigate the metabolism of oxycodone, oxymorphone and noroxycodone, three different pediatric cryopreserved hepatocytes preparations were incubated with oxycodone, noroxycodone and oxymorphone. All three pediatric human cryopreserved hepatocytes catalyzed the formation of noroxycodone, oxymorphone and noroxymorphone from oxycodone, where noroxycodone was shown to be the major metabolite. The formation of noroxycodone and oxymorphone from oxycodone was linear up to 2 hours in these hepatocytes. The rate of noroxycodone formation in 9-month old infant hepatocytes is nearly 3 and 9-times higher than the 6-day infant and 42-year old adult hepatocytes. A nearly 4-5-fold variation in the relative amounts of noroxycodone and oxymorphone was observed in three pediatric human cryopreserved hepatocytes. The amount of noroxycodone, oxymorphone and noroxymorphone formation in adult human cryopreserved hepatocytes are 1330.1, 118.3 and 165.6 pmol/ hr/ 10^6 cells, respectively, and these values are well within the range of similar values in pediatric cryopreserved hepatocytes (Figure 9). These results suggest that the enzymes responsible for oxycodone metabolism are equally expressed in pediatric populations compared to the adult. The formation of oxymorphone glucuronide in pediatric human cryopreserved hepatocytes was detected using LC-MS/ MS, however, because of non-availability of an authentic reference standard of oxymorphone glucuronide, the rate of its formation in these samples was not measured

Incubation of oxymorphone with three pediatric and one adult human cryopreserved hepatocytes resulted in the formation of noroxymorphone and oxymorphone glucuronide. And the incubation of noroxycodone with three pediatric and one adult human cryopreserved hepatocytes resulted in the formation of noroxymorphone. Under the experimental conditions used no noroxycodone or oxycodone glucuronides were detected using LC-MS/ MS. Unfortunately, an interfering peak at approximately 2 minutes was observed in all the pediatric and adult human cryopreserved hepatocytes incubated samples on reverse-phase HPLC. Hence, the detection or the relative quantitation of oxycodone, noroxycodone and oxymorphone glucuronide peaks in these samples was unsuccessful.

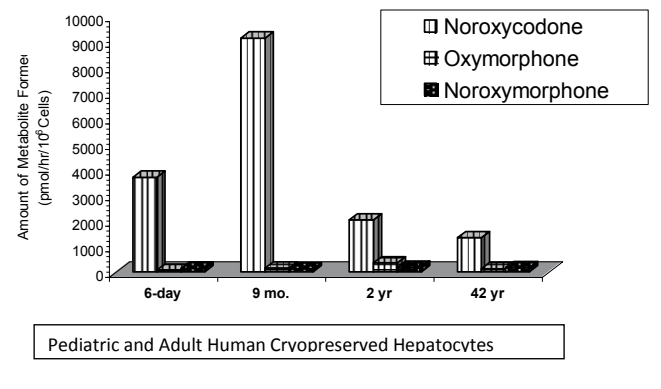

Figure 9: Comparative Metabolism of Oxycodone in Pediatric and Adult Human Cryopreserved Hepatocytes.

The incubations of oxycodone in the absence of various hepatocytes (negative control incubations), resulted in no formation of any oxycodone metabolites. These results suggest that the formation of oxidative and conjugative metabolites from oxycodone is catalyzed by the respective enzymes in the hepatocytes.

CONCLUSIONS

The results from this study show that liver microsomes isolated from various pediatric populations were able to catalyze the formation of noroxycodone and oxymorphone from oxycodone at variable rates. The formation of noroxymorphone was also detected in some pediatric liver microsomes. As observed in pediatric liver microsomes, adult human liver microsomes converted oxycodone to noroxycodone, oxymorphone and noroxymorphone. Noroxycodone was shown to be the major *in vitro* metabolite of oxycodone in all the pediatric populations and in adult human liver microsomes. In adults, N-demethylation of oxycodone to noroxycodone represents the predominant oxidative pathway in humans and was almost exclusively catalyzed by CYP3A4 [13]. CYP3A7 is a member of the CYP3A subfamily and is uniquely expressed in human fetal livers and has also been shown to be responsible for noroxycodone formation. It is detectable as early as 50 to 60 days of gestation with continued significant levels of expression through the perinatal period. The expression begins to decline after the first postnatal week, reaching non-detectable levels in most individuals by 1 year of age. Hepatic CYP3A4 expression begins to increase at about 1 week of age, reaching 30% of adult levels by 1-month [19]. Because of the simultaneous decline in CYP3A7 and increase in CYP3A4, total CYP3A protein expression over the entire developmental period remains constant [19,20]. These results suggest that elimination of noroxycodone, which is a major metabolite of oxycodone, is not limited in pediatrics.

Although, oxymorphone is an active metabolite, it is formed at very low levels in both pediatrics and adults. CYP2D6 is the major CYP-isoform responsible for oxymorphone formation. CYP2D6 protein and mRNA are present in fetal livers at less than 30 weeks of gestational age [21]. CYP2D6 protein levels increase significantly after birth, and continued upward to levels of about 50 – 75% of adult levels during the neonatal period. These results suggest that although oxymorphone is formed at low levels, its formation in pediatrics is not limited by the expression of CYP2D6.

At present, the UGT isoform(s) responsible for the glucuronidation of oxycodone, noroxycodone and oxymorphone in adult or pediatric liver microsomes have not been identified. However, the ability of pediatric liver microsomes and hepatocytes to form glucuronides confirms the expression of UGT(s) in various pediatric populations. The expression of UGTs in fetal and pediatric populations has been shown to be present [20]. However, UGT1A1, primarily responsible for bilirubin metabolism and UGT1A6 responsible for acetaminophen glucuronidation are shown to be absent in the fetus [22]. However, both UGT1A1 and UGT1A6 are shown to increase slightly in neonates [23]. The formation of oxymorphone glucuronide in pediatric and adult cryopreserved hepatocytes was confirmed using LC-MS/MS and HPLC methods. These results suggest that although oxymorphone is an active metabolite that is formed at low levels, its conjugation to form oxymorphone glucuronide is not limited in pediatrics.

Overall, liver microsomes and hepatocytes isolated from various pediatric age groups catalyzed oxycodone to both oxidative (Phase I) and conjugative (Phase II) metabolites, which is similar to adult human liver microsomes and hepatocytes (Figure 1). As in adults, a significant inter-individual variability in oxycodone metabolism was observed in various pediatric age groups. In pediatrics, the relative amounts of formation of oxycodone metabolites are different in various age groups, but the total metabolic profile of oxycodone in pediatrics appears to be similar to adults. The dose of oxycodone must be personalized individually in the clinic depending on their age.

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