METABOLISM OF PHENCYCLIDINE BY HUMAN LIVER MICROSOMES

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ABSTRACT:
These studies examined in vitro metabolism of phencyclidine (PCP) in a series of human liver microsomes (N = 10). Each sample was characterized for cytochrome P450 (CYP) content and for CYP1A, CYP2A, CYP2C, CYP2D, CYP2E, CYP3A, CYP4A, and lauric acid 11-hydroxylation metabolic activities. At least five PCP metabolites (c-PPC, t-PPC, PCHP, an unknown metabolite, and an irreversibly bound metabolite) were formed by the various human liver microsomes. Nevertheless, there was a large degree of interindividual variation in the metabolite formation. For example, the irreversibly bound metabolite was formed in detectable amounts in only four of the ten samples. c-PPC, t-PPC and the irreversibly bound PCP metabolite formation rates significantly correlated with CYP3A activity. The CYP3A inhibitor troleandomycin was used to inhibit the formation of PCP metabolites. Troleandomycin inhibition was dose dependent with the highest dose producing complete inhibition of the formation of c-PPC, t-PPC, PCHP, and the irreversibly bound metabolite. In addition, PCP inhibited CYP3A-mediated testosterone 6β-hydroxylation by 50%. Furthermore, the relative intensity of CYP3A immunoreactive proteins significantly correlated with testosterone 6β-hydroxylation and with PCP metabolite formation (except for the unknown metabolite). PCHP formation also correlated with CYP1A activity, while the formation of the unknown PCP metabolite correlated with CYP2A activity. These studies suggest that several CYP isozymes contribute to PCP metabolism and that CYP3A plays a major role in PCP bio-transformation in human liver microsomes.

Phencyclidine (PCP)2 was originally developed by Parke-Davis in the mid-1950’s for use as an anesthetic in humans but because of significant side effects it was never sold for human use. Nevertheless, in the 1960’s and 1970’s it became a popular drug of abuse. In addition to its anesthetic and analgesic effects in humans, PCP is known to produce a dose-dependent psychosis that resembles schizophreria with behavior described as extremely agitated, bizarre, unpredictable, and paranoid (1–3). There is also a long-lasting PCP-induced psychosis that appears to be an idiosyncratic reaction to which only certain individuals are susceptible (1, 3). The cause of this PCP-induced psychosis is unknown, but changes in the affinity or density of PCP and dopamine receptors (4), autoimmune mechanisms (5, 6) and differences in PCP metabolism and/or PCP metabolite irreversible binding to critical neurological macromolecules (7, 8) are among the most often mentioned explanations.

Cook et al. have shown that the elimination t1/2 of PCP varies considerably in humans, ranging from 7 to 57 hr (9–11). The observed values in t1/2 show a bimodal distribution. Although the majority of individuals have an average elimination of t1/2 ~17 hr, 2 of the 16 individuals in these studies had t1/2 values greater than 50 hr (11). These data suggest differences in PCP metabolism could help explain individual variations in PCP response and the reason for long-lasting effects in some individuals.

Oxidation by cytochrome P450 (CYP) enzymes is an important determinant in the overall clearance of many drugs, and differences in the CYP metabolic capabilities of humans is well documented (12, 13). Indeed, deficiencies and/or increases in tissue concentrations of some CYP isozymes can lead to unexpected toxicities and/or altered pharmacological effects of various drugs and xenobiotics (14, 15).

PCP metabolism and irreversible binding of metabolites have been studied extensively in various tissues of rat and rabbit (e.g. 16, 17); however, relatively little is known about human PCP metabolism except for the pharmacokinetic studies by Cook et al. (9, 10). Since metabolism is the major mechanism for termination of the pharmacological effects of PCP in animals (18, 19) and large differences in the terminal elimination half-life of PCP in humans (9, 10), we investigated PCP human metabolism to help understand the reasons for the individual differences in PCP response and pharmacokinetics. In these studies we examined the metabolism of PCP using a series of human liver microsomal samples, identified some of the PCP metabolites formed, and identified potential CYP isozymes involved in PCP metabolism.

Materials and Methods

Materials. Phencyclidine hydrochloride (PCP), cis-1-(1-phenyl-4-hydroxyphenoxy)cyclohexyl)piperidine (c-PPC), trans-1-(1-phenyl-4-hydroxyphenoxy)cyclohexyl)piperidine (t-PPC), 1-(1-phenylcyclohexyl)4-hydroxyphenoxy)cyclohexyl)piperidine (PCHP), 5-[N-(1-phenylcyclohexyl)amino]pentanoic acid (PCHAP) and 1-(1-[phenyl-3-2H]-cyclohexyl)piperidine ([3H]PCP, 15.69 Ci/mmole labeled at a metabolically stable site) were obtained from the National Institute on Drug Abuse (Rockville, MD). All other chemicals were purchased from Fisher Scientific (Springfield, NJ) unless otherwise stated. Trichloroacetic acid (TCA, 10%) was purchased from Baxter Scientific Products (Grand Prairie, TX), Troleandomycin (TAO), D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase,
times ranged from 5 to 120 min. The majority of the patients had serum PCP concentrations ranging from 0.10 – 0.62 μM after admission to an emergency room (28). These data showed that the concentrations of 216 patients who were diagnosed with acute PCP intoxication were obtained from Brandel-methylene-bis-acrylamide were purchased from U.S. Biochemical Corp. (Cleveland, OH). The GF/B filters and filtration device (Model M24R) for determination of PCP metabolite covalent binding were obtained from Brandel Laboratories (Gaithersburg, MD). C18 preparative columns (3 ml, Bakerbond spe, high hydrophobic octadecyl) were purchased from J.T. Baker (Phillipsburg, NJ). The μBondapack C18 steel column (10 μm, 3.9 × 300 mm) was obtained from Waters (Milford, MA). Silica alumina TLC sheets were purchased from EM Separations. The anti-rat CYP3A1/2 antiserum was purchased from Bio-Rad Laboratories (Hercules, CA). The anti-rabbit IgG (whole molecule) alkaline phosphotase conjugate were purchased from Upstate Biotechnology, Inc. using the following selective substrates for CYP enzymes: 7-ethoxyresorufin O-dealkylation (CYP1A2 (20)), caffeine N-demethylation (CYP1A2 (21)), coumarin 7-hydroxylation (CYP2C9 (23)), dextromethorphan O-demethylation (CYP2D6 (24)), chloroxazone 6-hydroxylation (CYP2E1 (25)), testosterone 6β-hydroxylation (CYP3A4 (26)), lauric acid 12-hydroxylation (CYP4A2 (27)) and lauric acid 11-hydroxylation (unknown isozyme).

In vitro Metabolic Incubations. Before determination of in vitro PCP metabolism by human liver microsomes, experiments were performed to determine the effect of microsomal protein concentration and reaction time. The incubation mixtures (final volume 0.5 ml) consisted of microsomal protein (0.1–2 mg/ml) and a NADPH regenerating system consisting 8 mM glucose-6-phosphate, 1 unit/ml glucose-6-phosphate dehydrogenase, 4 mM MgCl2 with the presence or absence (for the controls) of 5 mM NADP+. This mixture was pre-incubated at 37°C for 5 min. The reaction was initiated by the addition of PCP (1 μM) and [3H]-PCP as a tracer. Incubation times ranged from 5 to 120 min. The 1 μM concentration of PCP was chosen based on the average serum concentrations of 216 patients who were diagnosed with acute PCP intoxication after admission to an emergency room (28). These data showed that the majority of the patients had serum PCP concentrations ranging from 0.10–0.62 μM (or 25–150 ng/ml), but some individuals had >2.1 μM (or >500 ng/ml). Although no data is available for human PCP tissue to serum ratios, these data are available in the rat (29). In rats administered a dose of 1.3 mg/kg of PCP, a representative serum concentration at 15 min after dosing is 0.74 μM (or 180 ng/ml). At this same time, liver concentrations are nearly 9 times higher (i.e. 6.7 μM). Therefore, assuming similar liver tissue to serum ratios in humans, when serum concentrations are 0.1–0.62 μM in humans, the liver concentrations should be about 0.9–5.6 μM.

HPLC Analysis of PCP and Metabolites. Following the metabolic incubations, the PCP metabolites were immediately extracted from the incubation mixture. A 350 μl aliquot of the mixture, along with the internal standards of PCP and metabolites, was passed over a solvent-conditioned 3 ml C18 column. Prior to adding the metabolic incubation mixture, the extraction column was conditioned with 2.5 ml of 5% CH3CN containing 0.1% trifluoroacetic acid and 1% (CH3)3N. After adding the samples, the column was washed twice with 2.5 ml of conditioning buffer. PCP and the metabolic products formed were then eluted with two 2.5-ml washes of 100% CH3CN containing 0.1% trifluoroacetic acid and 1% (CH3)3N. The eluted PCP and metabolites were taken to dryness by vacuum centrifugation and were resuspended in 120 μl of 15% CH3CN/H2O containing 0.1% trifluoroacetic acid and 0.1% (CH3)3N. PCP and metabolites (100 μl per injection) were separated by HPLC using a μBondapack C18 steel column. A linear gradient from 15–20% CH3CN/H2O containing 0.1% trifluoroacetic acid and 0.1% (CH3)3N was held for 3 min, followed by a 20–35% gradient for the next 4 min, and isocratic conditions of 35% for the next 8 min. The flow rate was 2 ml/min. The Waters HPLC system consisted of Millennium chromatography software, a model 600E multisolvent delivery system, a model 717 autoinjector, and a model 486 tunable absorbance detector. Twenty sec-fractions were collected and analyzed for radioactive PCP and metabolites by liquid scintillation spectrometry. Analytical recoveries and identity of the metabolic products were determined by comparison with an exact amount of authentic external standards injected at the start of each analytical run. The elution of external and internal standards was monitored at 254 nm. This procedure could separate PCP and six known PCP metabolites. Since we did not detect two of these metabolites in preliminary experiments, they were not included in the analysis. These two minor dihydroxylated metabolites were: cis-4-(4’-hydroxyxipiperidino)-4-phenylcyclohexanol and trans-4-(4’-hydroxyxipiperidino)-4-phenylcyclohexanol. The four major metabolites used in this study and their HPLC retention times were c-PPC at 3.9 min, t-PPC at 6.05 min, PCHP at 7.4 min, PCAP at 8.6 min, and PCHAP at 9.5 min (see fig. 1). Because of the apparent covalent type bond of the irreversibly bound PCP metabolite, it had to be detected by trapping microsomal proteins on glass fiber filters after TCA precipitation of the proteins (see next section).

Determination of PCP Metabolite Irreversible Binding. The method for determination of in vitro metabolite irreversible binding was as previously described (30, 32). Briefly, duplicate 50 μl aliquots were removed from the incubation mixtures and the microsomal proteins were precipitated by addition of 2 ml ice cold 10% TCA and incubation on ice for 1 hr. The precipitated aliquots were then passed over GF/B filters and extensively washed with additional volumes of 10% TCA and 40% ethanol until no more radioactivity

<table>
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<th>Gender</th>
<th>Age (yr)</th>
<th>Race</th>
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<th>Alcohol Use</th>
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FIG. 1. Structure of PCP and three phase I metabolites in this study which were identified and quantitated by HPLC analysis.

TABLE 1
Donor information for human microsomal preparations

<table>
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<tr>
<th>Sample</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>Race</th>
<th>Smoker</th>
<th>Alcohol Use</th>
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<tr>
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<td>Male</td>
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<tr>
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<tr>
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<td>52</td>
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could be removed. The filters were air dried and the amount of metabolite irreversible binding was determined by liquid scintillation spectrometry analysis of the filters.

**In Vitro CYP Inhibition Experiments.** For substrate inhibition experiments, TAO, a selective human CYP3A inhibitor (31, 33), was used. The TAO was dissolved in methanol (final concentration of methanol in the incubation mixture was <0.05%). Varying concentrations of TAO were added just prior to addition of the components of the NADPH regenerating system and the mixtures were incubated at 37°C for 5 min. PCP was added and the reaction mixtures were incubated at 37°C for 20 min.

A method similar to the procedure used by Waxman et al. (34) was used for the testosterone metabolism experiments. Testosterone was dissolved in methanol and aliquoted into tubes, and the methanol was allowed to evaporate. The amount of testosterone added to the tubes equaled a concentration of 100 μM (440,000 DPM) in the final reaction volume. In separate tubes, varying amounts of PCP were combined with human liver microsomal protein (1 mg/ml) and the NADPH regenerating system (final volume 80 μl). This mixture was preincubated at 37°C for 5 min. The mixture was then transferred to the tubes containing testosterone and the reaction was carried out at 37°C for 10 min. The reactions were terminated by pipetting 5 μl of each mixture onto separate lanes of a silica gel TLC plate. The plates were allowed to dry and unlabeled testosterone and testosterone metabolite standards were then added to the lanes. The TLC solvent system consisted of 80% methylene chloride and 20% acetone. The plates were subjected to two consecutive TLC solvent separation steps to enhance the separation of the metabolites. The radioactivity on the plates was then analyzed by autoradiography. Areas on the plate corresponding to authentic testosterone and the 6β-hydroxylated testosterone metabolite were cut out and analyzed by liquid scintillation spectrometry.

**Western Blot Analysis of CYP3A.** Duplicate samples (10 μg/lane) of human liver microsomes were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis on a standard size 8% polyacrylamide gel for 5 hr at ~35 mAmp. The proteins were transferred to nitrocellulose filters at 12 V overnight at 4°C using prechilled 25 mM Tris, 192 mM glycine, 20% v/v methanol (pH 8.3) transfer buffer. The nitrocellulose filter was incubated for 2 hr in BSA blocking buffer (3% w/v BSA in TBS containing 0.05% v/v Tween-20) to reduce nonspecific binding and then washed twice for 5 min in TBS containing 0.05% Tween-20. Anti-rat CYP3A1/2 was diluted in antibody buffer (1% w/v gelatin in TBS containing 0.05% v/v Tween-20) and added to the filters at a final titer of 1:4,000. Characterization of this antibody is published elsewhere (35, 36). The filters were incubated overnight at room temperature with gentle mixing. Filters were washed as previously described and a goat anti-rabbit IgG alkaline phosphotase conjugate (1:20,000) was added. After incubation at room temperature for about 2 hr, the filter was washed and protein bands were detected with Bio-Rad color development reagents following the manufacturer’s directions. The relative amount of anti-CYP3A reactive protein was measured using a Bio-Rad GS-670 imaging densitometer. As part of the validation of this assay, we first established there was a linear relationship between amount of rat liver microsomal protein (range of 0.25 to 20 μg/lane) applied to the gel and the relative band intensities. The correlation coefficient for this relationship was 0.987 (p < 0.05).

**Data Analysis.** Linear regression analysis was used for determining the relationship between PCP metabolism and CYP metabolic activity. It was also used for determining the relationship between PCP metabolism and relative intensity of CYP3A immunoreactivity on Western blots. An ANOVA followed by Dunnett’s multiple range test were used for comparing control and treatment groups. All statistical analyses were performed using SigmaStat for Windows (Jandel Scientific Software, San Rafael, CA). Statistical significance was defined at a level of p < 0.05.

**Results**

**In Vitro Formation of PCP Metabolites by Human Liver Microsomes.** Formation of PCP metabolites was dependent on the presence of NADPH, protein concentration, and was linear for at least 40 min (data not shown). Based on these experiments, a protein concentration of 1 mg/ml and an incubation time of 20 min were chosen as optimal. At least five PCP metabolites (c-PPC, t-PPC, PCHP, an unknown PCP metabolite, and an irreversibly bound PCP metabolite) were formed at significant levels by the human liver microsomal samples, depending on the human liver sample (figs. 2 and 3). The identity of c-PPC, t-PPC and PCHP was based on co-elution with external standards. The PCHAP metabolite was not detected. An additional radioactive peak eluted from the HPLC column just after the PCHP external standard but before PCHAP. Since it did not co-elute with any of known metabolite standards and since the radio-label on the [3H]-PCP was at a metabolically stable site, we assumed it was a PCP metabolite. No attempt was made to determine its identity. There was a large degree of inter-individual variation in the ability of the human liver microsomal samples to form some of the PCP metabolites (figs. 2 and 3). There was no evident correlation between the variation in PCP metabolism and the age, gender, or drug history of the donors. The formation rate of c-PPC varied 36-fold between individuals. The variation in t-PPC and PCHP formation rates were 20- and 10-fold, respectively. There was only a 3-fold variation in the ability to form the unknown metabolite. The irreversibly bound PCP metabolite was formed at appreciable amounts only in human liver samples 2, 6, 9, and 11 (fig. 3).

**Correlation of PCP Metabolite Formation with CYP Isoform Activity.** The formation rates of each of the PCP metabolites were correlated with each of the characterized CYP activities listed in the experimental procedures. The PCP metabolite formation rates that significantly correlated with the CYP activities are shown in table 2. The formation rates of c-PPC, t-PPC and PCHP were correlated with CYP3A activity, while CYP2A activity correlated with the formation of the unknown PCP metabolite. In addition, formation of c-PPC, t-PPC and the irreversibly bound PCP metabolite correlated with CYP3A-mediated metabolism.

**In Vitro Inhibition of PCP Metabolite Formation by TAO.** Because CYP3A appeared to play a major role in PCP metabolism, we tested the ability of TAO to inhibit the formation of PCP metabolites. Although TAO is a selective inhibitor of the CYP3A family, it is not specific for a single CYP3A isozyme (31, 33). Because human liver microsomal samples 2, 6, and 11 had intermediate to high CYP3A activities (fig. 4) and intermediate to high PCP metabolite formation rates, these microsomal samples were chosen for further analysis. Although human liver sample 9 also had an intermediate rate of PCP metabolism, this sample was not included in this particular experiment because the microsomes were obtained from an infant. We
PCP (1 μM and a tracer dose of [3H]-PCP) were incubated with human liver microsomal protein (1 mg/ml) for 20 min at 37°C in the presence of a complete NADPH regenerating system. PCP metabolite irreversible binding was determined as described in experimental procedures. All values were corrected for control incubations without NADP.

Table 2

Correlation of PCP metabolite formation with CYP isoform activities in human liver microsomal samples

<table>
<thead>
<tr>
<th>PCP Metabolite</th>
<th>CYP isoform that Correlates with PCP Metabolite Formation</th>
<th>Correlation Coefficient (r)</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>c-PPC</td>
<td>CYP3A</td>
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<td>0.011</td>
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<tr>
<td>t-PPC</td>
<td>CYP3A</td>
<td>0.70</td>
<td>0.025</td>
</tr>
<tr>
<td>PCHP</td>
<td>CYP1A</td>
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<td>0.012</td>
</tr>
<tr>
<td>Unknown Metabolite</td>
<td>CYP2A</td>
<td>0.66</td>
<td>0.039</td>
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<td>Irreversibly bound PCP Metabolite</td>
<td>CYP3A</td>
<td>0.68</td>
<td>0.029</td>
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* N = 10 per group.

** CYP activities that did not show a significant correlation were CYP2C, CYP2D, CYP2E, CYP4A, and lauric acid 11-hydroxylation.

were concerned that the differential expression of CYP3A isozymes by fetal livers (37) could have added an unknown variable to the interpretation of these results. TAO inhibited the formation of c-PPC, t-PPC, PCHP and the unknown PCP metabolite in a dose-dependent fashion in these microsomes (fig. 5). The formation of all PCP metabolites was completely inhibited by TAO for the unknown metabolite, which was inhibited to ~75% of the control value. In addition, TAO completely inhibited the formation of the irreversibly bound metabolite in human liver samples 2, 6, and 11 (fig. 6). Human liver samples 2 and 9 were not included in this analysis because of their lower ability to form the irreversibly bound metabolite (see fig. 3).

In Vitro Inhibition of Testosterone 6β-hydroxylation by PCP. Since testosterone 6β-hydroxylation is considered to be a CYP3A specific reaction, the ability of PCP to inhibit this reaction was tested. PCP inhibited the 6β-hydroxylation of testosterone in a dose-dependent fashion, however the highest dose of PCP (1 mM) only produced a 50% inhibition in each of the samples (fig. 7).

Correlation of PCP Metabolite Formation with CYP3A Protein Levels. To provide further evidence that CYP3A plays a role in PCP metabolism, PCP metabolite formation rates were correlated with the relative CYP3A content of each of the human liver microsomal samples using linear regression analysis. Prior to this analysis, we also used linear regression to determine if testosterone 6β-hydroxylase activities (see fig. 4) correlated with CYP3A protein levels in the human liver microsomal samples. This analysis showed that the
Irreversible binding of PCP metabolite(s) was determined as described in experimental procedures. Human liver samples 6 and 11 were used in this analysis because they formed the largest amounts of the irreversibly bound metabolite (see fig. 3).

Experiments were performed as described in experimental procedures. Asterisks indicate values significantly different (p < 0.05) from controls. Human liver samples 2, 6, and 11 were used for this analysis because of their high levels of testosterone 6β-hydroxylase activity.

**TABLE 3**

Results from the correlation of PCP metabolite formation rates with relative intensity of CYP3A immunoreactivity protein in human liver microsomal samples

<table>
<thead>
<tr>
<th>PCP Metabolite</th>
<th>Correlation Coefficient (r)</th>
<th>P Value</th>
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<tbody>
<tr>
<td>c-PPC</td>
<td>0.77</td>
<td>0.015</td>
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<tr>
<td>t-PPC</td>
<td>0.83</td>
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<tr>
<td>PCHP</td>
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<td>0.015</td>
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<td>0.58</td>
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a N = 10 per group. Determined by Western blot analysis.

that are metabolized by the CYP3A isoforms (38).

In humans, the CYP3A family accounts for a major portion (~30%) of the total CYP content in liver (13). Three known functional isoforms (CYP3A4, CYP3A5, and CYP3A7) comprise the CYP3A family in humans (39). Of these forms, CYP3A4 appears to be the most abundant in adult human liver, while CYP3A7 appears to be the major fetal CYP (37). CYP3A5 is polymorphically expressed in humans, with only 30% of human livers expressing detectable levels (40, 41). In addition, although there was a large variation in the CYP3A content between individual subjects (as measured by CYP3A-mediated testosterone 6β-hydroxylase activity, fig. 4), these data correlated well with the amount of CYP3A immunoreactive protein in the microsomes (as measured by Western blot analysis). Indeed, linear regression analysis of the CYP3A catalytic activity vs. CYP3A protein content showed a significant correlation (r = 0.80). A large variation in CYP3A content between subjects has been reported by other investigators (13).

The formation of c-PPC, t-PPC, and the irreversibly bound PCP metabolite significantly correlated with testosterone 6β-hydroxylation, a reaction that is catalyzed primarily by CYP3A4 in humans (34, 41). To further investigate the role of CYP3A isoforms in PCP metabolism, substrate inhibition studies were conducted. For these studies, human liver samples 2, 6, and 11 were chosen because they had intermediate to high CYP3A (testosterone 6β-hydroxylase) activity and intermediate to high PCP metabolism rates (figs. 4 and 2, respectively). Despite the higher CYP3A activity in these three samples, only one donor had been treated with a prototypic phenobarbital-type CYP3A inducer (i.e. pentobarbital) (40, 42).

The selective CYP3A isozyme inhibitor, TAO (31, 33), completely inhibited the formation of c-PPC, t-PPC, PCHP, and the irreversibly bound PCP metabolite at 100 μM TAO (figs. 5 and 6). TAO inhibition of CYP3A catalyzed testosterone 6β-hydroxylation is reported to be specific even at concentrations of TAO much higher than used in this study (i.e. up to 400 μM TAO (31)). The complete inhibition of PCHP formation by TAO was somewhat surprising since PCHP formation rates did not significantly correlate with testosterone 6β-hydroxylation (a metabolic product of CYP3A). The cause of this discrepancy is not known. Nevertheless, the inhibition of PCHP formation by TAO is suggestive of CYP3A isozyme involvement in the formation of this metabolite.

The relative CYP3A content of each of the human liver samples (as determined by immunoblot) significantly correlated with the formation rates of c-PPC, t-PPC, and PCHP (table 3). The partial inhibition of testosterone 6β-hydroxylation by PCP also suggested that PCP could interact with a CYP3A isozyme (fig. 7). These data provide strong evidence that one or more member(s) of the CYP3A isozyme family mediate the formation of c-PPC, t-PPC, PCHP, and the irreversibly bound PCP metabolites. Furthermore, since these studies show CYP3A is a major metabolic pathway for PCP liver elimination and there are large in vivo variations in CYP3A liver expression and activity (as found in this study), this could be a reason for the large variations in the PCP elimination half-life (ranging from 7 to 57 hr) found in humans (9–11). However, it is also worth noting that phase II metabolism of PCP (primarily sulfonation and glucuronidation (16)) could also contribute to the variation in PCP half-life. Indeed, human liver sulfotransferase(s) are polymorphically expressed (43).

As previously mentioned, an unknown PCP metabolite was formed by the human liver microsomes. This metabolite did not co-elute with any of the authentic PCP metabolite standards. We did not attempt to determine the identity of this metabolite. Although the metabolite standards used in these studies were chosen because they are known to be major metabolites in several species (16), there are many other PCP metabolites which could be formed. For instance, 1-(1-phenyl-3-hydroxycyclohexyl)piperidine is formed by liver microsomes and has been detected in the urine of mice, monkeys, and humans (16, 44, 45). An authentic standard for this metabolite was unavailable.

While formation of the unknown PCP metabolite significantly correlated with CYP2A activity, it did not correlate with testosterone 6β-hydroxylation activity or with the relative CYP3A content of the human liver microsomal samples. However, formation of the unknown PCP metabolite was inhibited up to 80% by TAO (fig. 5). We do not have a good explanation for this seemingly conflicting result.

The PCP metabolites formed by human liver microsomes in this
study are consistent with previous reports (16, 17) with the exception of the irreversibly bound PCP metabolite. To our knowledge, our studies are the first to demonstrate the formation of the irreversibly bound PCP metabolite in human liver microsomes. Irreversibly bound PCP metabolites are known to be formed by rat liver microsomes (7, 46, 47), as well as by rabbit lung and liver microsomes (48, 49, 50). Shelnutt et al. (32) have recently shown that the formation of this metabolite in normal rats is found only in the males and that the male-specific CYP2C11 isoform is responsible for (or at least involved in) its formation. The toxicological or pharmacological consequences of PCP metabolite irreversible binding are not known, and there are no reports of liver toxicity as a result of PCP use. Since CYP3A appears to play a role in the formation of the irreversibly bound PCP metabolite and CYP3A mRNA has been detected in human brain tissue (51), it is interesting to speculate that the formation of an irreversibly bound metabolite in the brain could play a role in the idiosyncratic psychotic reactions associated with PCP use. Further studies are needed to determine if this irreversibly bound PCP metabolite is formed in the human brain.

In conclusion, this study demonstrated that there are large inter-individual differences in the ability of human liver microsomes to metabolize PCP. In addition, members of the CYP3A family appeared to play a major role in the biotransformation of PCP. Since CYP3A isoforms are known to be variably and polymorphically expressed (13, 40), variability in human microsomal metabolism of PCP should be expected. Differences in pharmacological and/or toxicological response to drugs and other xenobiotics as a result of differences in CYP expression are well known (15). While there appears to be a large degree of variability in the ability of humans to metabolize PCP, the precise role of metabolism by CYP enzymes in mediating protective or toxic effects of PCP remains to be determined.

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References


