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NOVEL METABOLITES OF BUPRENORPHINE DETECTED IN HUMAN LIVER MICROSOMES AND HUMAN URINE

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ABSTRACT:

The in vitro metabolism of buprenorphine was investigated to explore new metabolic pathways and identify the cytochromes P450 (P450s) responsible for the formation of these metabolites. The resulting metabolites were identified by liquid chromatography-electrospray ionization-tandem mass spectrometry. In addition to norbuprenorphine, two hydroxylated buprenorphine (M1 and M2) and three hydroxylated norbuprenorphine (M3, M4, and M5) metabolites were produced by human liver microsomes (HLMs), with hydroxylation occurring at the *tert*-butyl group (M1 and M3) and at unspecified site(s) on the ring moieties (M2, M4, and M5). Time course and other data suggest that buprenorphine is *N*-dealkylated to form norbuprenorphine, followed by hydroxylation to form M3; buprenorphine is hydroxylated to form M1 and M2, followed by *N*-dealkylation to form M3 and M4 or M5. The involvement of selected P450s was investigated using cDNA-expressed P450s coupled with scaling models, chemical inhibition, monoclonal antibody (MAb) analysis, and correlation studies. The major enzymes involved in buprenorphine elimination and norbuprenorphine and M1 formation were P450s 3A4, 3A5, 3A7, and 2C8, whereas 3A4, 3A5, and 3A7 produced M3 and M5. Based on MAb analysis and chemical inhibition, the contribution of 2C8 was higher in HLMs with higher 2C8 activity, whereas 3A4/5 played a more important role in HLMs with higher 3A4/5 activity. Examination of human urine from subjects taking buprenorphine showed the presence of M1 and M3; most of M1 was conjugated, whereas 60 to 70% of M3 was unconjugated.

Buprenorphine, a semisynthetic derivative of the alkaloid thebaine (Lewis, 1973), is a partial μ-opioid agonist and κ-opioid antagonist (Cowan et al., 1977). It was first developed as an analgesic for moderate to severe pain in the early 1970s, but is currently more widely used as a replacement therapy for opiate dependence. Buprenorphine has comparable effects to methadone in regard to treatment of opiate-dependent patients (Strain et al., 1996; Johnson et al., 2000), but has reduced risk because of the "ceiling effect" associated with its partial μ-opioid agonist properties (Walsh et al., 1994, 1995).

Absorption, distribution, metabolism, and excretion studies of buprenorphine have been carried out in humans using gas chromatography-mass spectrometry (Cone et al., 1984), and in animals using thin-layer chromatography of tritiated buprenorphine (Brewster et al., 1981; Pontani et al., 1985). These studies suggested that buprenorphine was mainly metabolized by *N*-dealkylation and glucuronidation of both buprenorphine and norbuprenorphine. A tentative 6-*O*-demethyl norbuprenorphine in free and conjugated form was observed in rat urine (Pontani et al., 1985), and some unknown polar metabolites were found in rat bile samples (Brewster et al., 1981). No evidence was given for additional metabolites in humans (Cone et al., 1984). However, a recent study by Picard et al. (2005) using liquid chromatography-tandem mass spectrometry identified the presence of two hydroxylated metabolites, one of buprenorphine and one of norbuprenorphine, in human liver microsomes (HLMs) and urine samples from patients treated with buprenorphine. Buprenorphine *N*-dealkylation is mainly catalyzed by cytochrome P450 (P450) 3A4 (Iribarne et al., 1997; Kobayashi et al., 1998), with involvement of P450 3A5 and 2C8 (Moody et al., 2002; Picard et al., 2005). The involvement of specific P450s in production of the hydroxylated metabolites was limited to a finding that trace amounts of hydroxy-buprenorphine were produced by P450 3A4-, 3A5-, and 3A7-transfected cell lines (Picard et al., 2005).

In our previous study, we observed a higher rate of buprenorphine elimination than of norbuprenorphine formation in HLMs, suggesting that there might be some other routes for metabolism of buprenorphine or its metabolites (Chang and Moody, 2005). In this paper, we report a study of the metabolism of buprenorphine in HLMs and analysis of human urine from subjects treated with buprenorphine. The identification of new metabolites was achieved by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS), and the involvement of P450s in the formation of new metabolites was clarified using cDNA-expressed human P450s and correlations with a panel of HLMs. The contribution of each enzyme was estimated by inhibitory analysis using monoclonal antibodies

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ABBREVIATIONS: HLM, human liver microsome; P450, cytochrome P450; MAb, monoclonal antibody; NADPH GS, NADPH generating system; LC-ESI-MS/MS, liquid chromatography-electrospray ionization-tandem mass spectrometry; MS, mass spectrometer; SRM, selected reaction monitoring; SIM, selected ion monitoring; CID, collision-induced dissociation; RAF, relative activity factor.

(MAbs) and chemical inhibitors in phenotyped HLMs, and also was predicted by relative activity factor (RAF) and immunoquantification scaling approaches. Based on our results, an extended biotransformation profile is proposed for buprenorphine.

Materials and Methods

Materials. Buprenorphine (for incubation), D-glucose 6-phosphate monosodium salt, glucose-6-phosphate dehydrogenase, β-NADP sodium salt, EDTA disodium salt, $MgCl_2$, β-glucuronidase (from *Helix pomatia*, which also has sulfatase activity), trimethoprim, 8-methoxypsoralen, sulfaphenazole, and quinidine were obtained from Sigma-Aldrich (St. Louis, MO). Furafylline was obtained from Synergy House (Manchester, UK). Buprenorphine (for analysis), d_4-buprenorphine, norbuprenorphine, and d_9-norbuprenorphine were purchased from Cerilliant (Round Rock, TX). Ketoconazole was obtained from ICN Biomedicals Inc. (Aurora, OH). 10-Hydroxybuprenorphine, buprenorphine *N*-oxide, and 10-oxobuprenorphine were provided by Reckitt Benckiser Healthcare Limited (Hull, UK). The liver samples were obtained from Tissue Transformation Technologies (Edison, NJ). Insect cell cDNA-expressed human P450s (Supersomes) and 15 phenotyped HLMs were purchased from BD Gentest (Woburn, MA). Inhibitory MAbs to human P450 3A4/5 and 2C8 were provided by the National Cancer Institute of the National Institutes of Health (Bethesda, MA). All aqueous reagents were prepared in purified water (specific resistance >18.2 mΩ/cm) obtained by a Milli-Q Plus water purification system (Millipore, Billerica, MA).

In Vitro Incubations of Buprenorphine with HLMs. Microsomes were prepared from human liver by differential centrifugation as described by Nelson et al. (2001). The first centrifugation was at 9000*g*; the homogenization buffer contained 0.25 M sucrose, and 10 strokes of homogenization were used. HLMs prepared in our laboratory are not thoroughly phenotyped; to enhance the probability of having a representative amount of different P450 enzymes, pooled HLMs ($n = 5$) were used for initial metabolite identification studies. The incubation mixture (final volume 500 μl) contained incubation buffer (0.1 M phosphate buffer, pH 7.4 with 1.0 mM EDTA and 5.0 mM $MgCl_2$), a NADPH-generating system (NADPH GS) composed of 10 mM glucose 6-phosphate, 1.2 mM NADP, and 1.2 units of glucose-6-phosphate dehydrogenase, 0.5 mg/ml microsomal protein, and 10 μM buprenorphine or norbuprenorphine. The reaction was initiated by adding the NADPH GS and incubated at 37°C in a shaking water bath for the specified times. For qualitative studies, after a 30-min incubation, the mixture was adjusted to pH >10 with 50 μl of 1 N NaOH, followed by extraction with a mixture of *n*-butyl chloride and acetonitrile (4:1, v/v). For quantitative studies, the reaction was terminated by the addition of 200 μl of ice-cold methanol, and the samples were stored at −75°C until analysis.

In Vitro Incubations of Buprenorphine with Recombinant Human P450s. The metabolism of buprenorphine and norbuprenorphine was evaluated in microsomes prepared from insect cells transfected with cDNAs encoding for human P450s 1A2, 2A6, 2B6, 2C8, 2C9*1, 2C18, 2C19, 2D6*1, 2E1, 3A4, 3A5, and 3A7. Supersomes that coexpressed cytochrome b_5 were used where available; this was not the case for 1A2, 2C18, and 3A5. Buprenorphine or norbuprenorphine (10 μM) was incubated at 37°C for 20 min with 25 pmol of P450 in the incubation buffer described above. Control insect cell microsomes were used at the mean protein concentration averaged over all of the Supersomes. All reactions were initiated by addition of the NADPH GS and stopped by the addition of 200 μl of ice-cold methanol, after which the samples were stored at −75°C until analysis.

Inhibition of Buprenorphine Metabolism Using MAbs. The role of P450 3A4/5 and 2C8 was measured by the addition of the P450 target-specific MAb, either alone or in combination, to the reaction mixture, using the procedure proposed by Yang et al. (1999). The recommended volumes (10 μl) of MAbs specific for P450 3A4/5 or 2C8 were mixed with phenotyped HLMs in 0.5 ml of incubation buffer and preincubated for 5 min at 37°C. Tubes were then placed on ice, buprenorphine was added (final concentration 10 μM), and the reaction was initiated by addition of the NADPH GS. The reaction continued for specified times at 37°C and was terminated with 200 μl of ice-cold methanol. Ten microliters of egg lysozyme was used as a control.

Chemical Inhibition Studies. The effect of the selective P450 inhibitors on more extensive studies were performed in phenotyped HLMs using the selective P450 3A4/5 inhibitor ketoconazole (2 μM) (Newton et al., 1995; Sai et al., 2000) and the selective P450 2C8 inhibitor trimethoprim (100 μM) (Wen et al., 2002). The inhibitor and buprenorphine (final concentration 10 μM) were added to the reaction mixture, and the reaction was initiated by the addition of the NADPH GS in a 37°C shaking water bath. The reaction continued for specified times and was terminated by the addition of 200 μl of methanol. The incubation sample with no inhibitor served as control.

Correlation Studies. HLMs from 15 individual donors, along with data for P450-specific enzyme activities, provided by BD Gentest, were used to study the relationship between the metabolism of buprenorphine and the metabolism of selective P450 substrates. The ability of HLMs from each donor to metabolize buprenorphine was correlated with the P450-specific enzyme activities for each sample. The assay was performed with 10 μM buprenorphine and incubated for the specified times.

In Vivo Metabolism of Buprenorphine. Twenty-four-hour postdose urine samples were collected from seven subjects who had been maintained on a daily sublingual dose of 16 mg of buprenorphine for at least 21 days. A 1-ml aliquot of each urine sample was adjusted to pH 5 with sodium acetate buffer (0.1 M) and treated with 5000 units of β-glucuronidase (containing sulfatase). The mixture was incubated at 50°C for 16 h. Another aliquot of the urine samples was analyzed without hydrolysis. Blank urine samples also underwent hydrolysis to control for interference arising from endogenous materials.

LC-ESI-MS/MS Analysis. The quantification of buprenorphine and norbuprenorphine (or semiquantification of hydroxylated metabolites) in incubation samples and urine samples was performed using a modification of our previously described LC-ESI-MS/MS method (Moody et al., 2002). The incubation samples were made basic (pH >10) by the addition of 50 μl of 1 N NaOH and extracted with a 4-ml mixture of *n*-butyl chloride and acetonitrile (4:1, v/v); the organic layer was dried under N_2. The final residue was reconstituted to a volume of 75 μl using the initial mobile phase, and 20 μl was injected into the liquid chromatograph.

Mass spectrometric analysis was performed on a TSQ 7000 or TSQ Quantum spectrometer (Thermo Electron, San Jose, CA) equipped with a triple-quadrupole MS and an ESI source operated at 4.5 kV. The MS was set to scan for positive ions. Quantification was performed by selected reaction monitoring (SRM) transitions *m/z* 468 to *m/z* 396 (buprenorphine), *m/z* 414 to *m/z* 101 (for TSQ 7000) (norbuprenorphine), *m/z* 472 to *m/z* 400 (d_4-buprenorphine), and *m/z* 423 to *m/z* 110 (d_9-norbuprenorphine). The semiquantification of hydroxylated metabolites by SRM is described in detail under *Results*. MS/MS conditions used were 3.0 mTorr argon collision gas and 45 eV collision potential. When the Quantum was used, we found that norbuprenorphine had better sensitivity when the survivor molecular ion was monitored (i.e., 22 eV collision potential with *m/z* 414 to *m/z* 414) (Huang et al., 2006). The liquid chromatograph was a Hewlett-Packard Series 1100 HPLC (Agilent Technologies, Palo Alto, CA). The chromatographic separations were conducted on a 3 μM YMC ODS-AQ column (2.0 × 50 mm cartridge) (Waters, Milford, MA). The mobile phase was Milli-Q H_2O (A) and CH_3CN (B), both containing 0.1% formic acid. The gradient elution went from 97% A at 1 min to 80% A at 3 min, holding for 5 min, then decreased to 20% A at 10 min, holding for 2 min.

Qualitative studies were performed on an Inertsil C18 column (250 × 2.1 mm i.d.), packed with 3-μm particles (Metachem Technologies, Inc., Torrance, CA). Isocratic elution was performed at 81% A with a flow rate of 0.25 ml/min. The screening of metabolites by mass spectrometry was based on full-scan, selected ion monitoring (SIM), constant neutral loss scan, precursor ion scan, and product ion scan. The constant neutral loss scan of 54 u was used to detect the metabolites that undergo a loss of the cyclopropylmethyl group. The precursor ion scans of *m/z* 396 and *m/z* 101 were used to detect the metabolites that can produce typical fragment ions at *m/z* 396 and *m/z* 101 under the collision-induced dissociation (CID) conditions. The product ion scan was used to identify the metabolites.

Results

In our previous studies on in vitro metabolism of buprenorphine, we focused on use of substrate concentrations that approached therapeutic

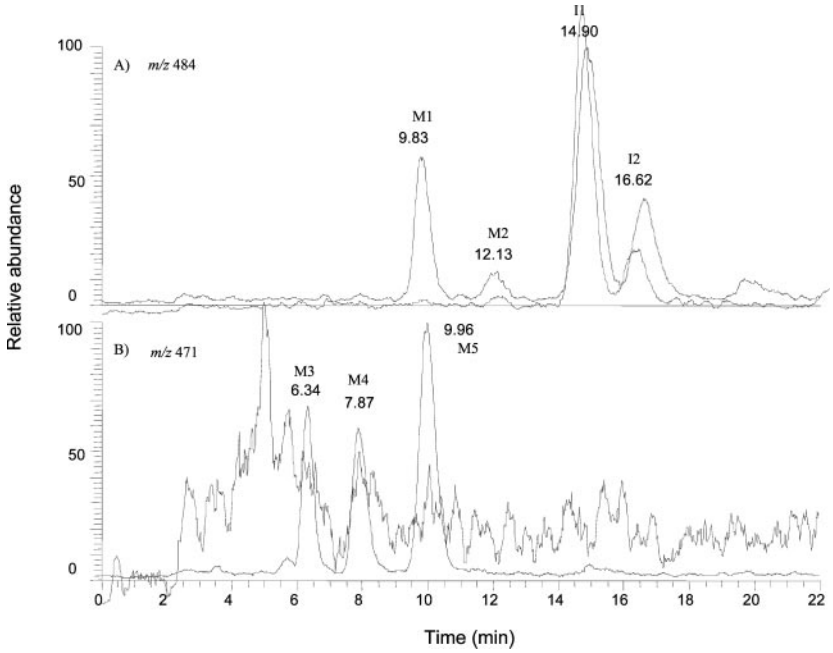

FIG. 1. The SIM chromatograms of the hydroxylated buprenorphine metabolites M1 and M2 (A) and the hydroxylated norbuprenorphine metabolites M3, M4, and M5 (B) after incubation of 10 μM buprenorphine for 30 min with HLM at 1.0 mg protein/ml. The dashed line is the control sample incubated with heat-inactivated HLMs.

Since the purpose of this study was to identify new metabolites, we have used a higher concentration, 10 μM, for in vitro experiments to enhance our ability to detect what might be minor metabolites. This concentration, which is less than the reported K_m for buprenorphine metabolism (Kobayashi et al., 1998), still meets the criterion suggested by Bjornsson et al. (2003) for P450 phenotyping studies. The in vivo relevance will be shown from studies in human urine.

Buprenorphine Elimination and Norbuprenorphine Formation in HLMs. When buprenorphine (10 μM) was incubated with pooled HLMs ($n = 5$), norbuprenorphine formation only accounted for 46% and 37% of buprenorphine elimination at 20 min and 60 min incubation time, respectively (data not shown). Higher buprenorphine elimination compared with norbuprenorphine formation suggested that other biotransformation pathways for buprenorphine or its metabolites exist in HLMs.

Mass Spectrometric Analysis of Buprenorphine. Under the CID-MS/MS conditions, the characterized product ions generated from protonated molecular ions of buprenorphine (*m/z* 468) were at *m/z* 414, *m/z* 396, and *m/z* 101 (Moody et al., 2002). A $[M - 54]^+$ peak at *m/z* 414 (referred to as the a-moiety) showed the removal of a cyclopropylmethyl group. The peak at *m/z* 396 (referred to as the b-moiety) was formed by combination of the loss of a methyl group and cleavage of a *tert*-butyl group instead of loss of the cyclopropylmethyl group and a water molecule, which was confirmed by the presence of a high, abundant product ion at *m/z* 400, produced from d_4-buprenorphine (*m/z* 472) (data not shown). This assignment was consistent with previous work reported by Polettini and Huestis (2001). At the low mass range, a fragment ion at *m/z* 101 (referred to as the c-moiety) was assigned to the alkyl side chain $HOC(CH_3)C(CH_3)_3^+$ at C-7, and it can lose a water molecule to form the fragment ion at *m/z* 83. Another fragment ion at *m/z* 55 corresponds to the cyclopropylmethyl group.

Identification of in Vitro Phase I Metabolites of Buprenorphine. In HLMs, the major metabolite, norbuprenorphine, formed by *N*-dealkylation of buprenorphine, has been studied in great detail. In the current study, different scan modes of the triple quadrupole MS were of 54 u and a precursor ion scan of *m/z* 396 and *m/z* 101 showed the presence of hydroxylated buprenorphine and norbuprenorphine. In initial experiments, norbuprenorphine was found to readily form an adduct ion with acetonitrile (plus 41 u) which shows better response on the mass spectrometer used than the protonated molecular ion. As such, the acetonitrile adduct ion was used to determine structurally related metabolites of norbuprenorphine. The *m/z* 484 and *m/z* 471 ions correspond to the hydroxylated buprenorphine protonated molecular ion and hydroxylated norbuprenorphine adduct ion with acetonitrile. There are four peaks in the SIM chromatogram at *m/z* 484 and three peaks at *m/z* 471 (Fig. 1). At retention times 9.83 min (M1) and 12.13 min (M2) (Fig. 1A), and retention times 6.34 min (M3), 7.87 min (M4), and 9.96 min (M5) (Fig. 1B), the peaks are absent in the chromatograms of the corresponding blank control samples. Peaks at retention times 14.90 min (I1) and 16.62 min (I2) in the SIM chromatogram of *m/z* 484 were also present in the control samples incubated with heat-inactivated microsomes, and their amounts did not change with changes in incubation time, suggesting that these two peaks are probably inert impurities.

When HLMs were incubated with buprenorphine, the microsomal protein precipitated with methanol, and the supernatant was directly injected into the LC-MS/MS, the same, and no additional, metabolites were observed. Selected ion monitoring of other possible metabolites, such as *O*-demethyl, *N*-oxide, and di-hydroxyl metabolites, showed negative results. The oxidative degradation compounds of buprenorphine found in sublingual tablets, i.e., 10-hydroxybuprenorphine, buprenorphine *N*-oxide, and 10-oxobuprenorphine, were not detected in microsomal samples using comparisons with the reference compounds. 6-*O*-Demethyl norbuprenorphine, which was tentatively identified in rat bile (Pontani et al., 1985), was not identified in HLMs.

The structure of the metabolites has been proposed by interpreting their product ion mass spectra and comparison with that of parent drug. The CID product ion mass spectrum of M1 (Fig. 2A) presented the strongest peak at *m/z* 396, suggesting that the b-moiety is intact. The *m/z* 414 ion in the CID product ion scan of buprenorphine shifted by 16 for M1 and, meanwhile, the *m/z* 55 ion was present, indicating

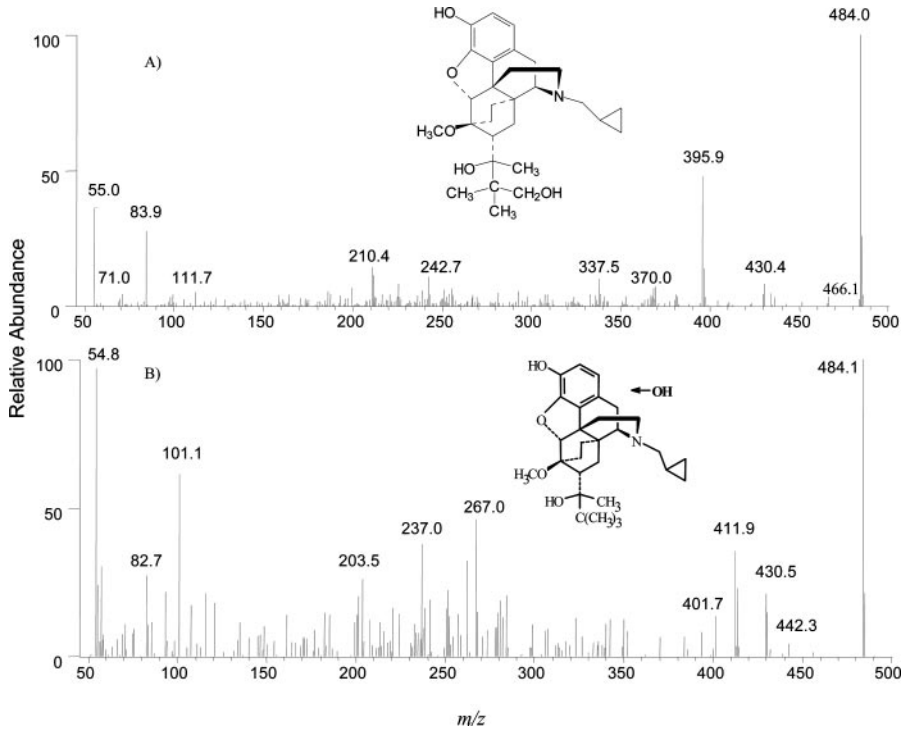

FIG. 2. CID product ion mass spectra of M1 (A) and M2 (B), and deduced structures.

occurred at the a-moiety. The absence of *m/z* 101 ion confirmed that the addition of a hydroxyl group was on the c-moiety. In consideration of the spatial hindrance and molecular stability, the hydroxylation occurred at the *tert*-butyl group.

The CID-MS/MS spectrum of M2 presents characteristic product ions at *m/z* 430 and *m/z* 412, and a strong fragment ion at *m/z* 101 (Fig. 2B). The presence of *m/z* 430 and *m/z* 101 ions suggests that the cyclopropylmethyl group and the alkyl side chain at C-7 position are intact; the addition of a hydroxyl group might occur at one of the ring moieties.

Three peaks were observed in the SIM chromatogram of *m/z* 471 (Fig. 1B). The characteristic fragment ion *m/z* 101 corresponding to the alkyl side chain at C-7 was absent in the product ion scan of M3 (Fig. 3A), whereas it was present in the product ion scan of M4 (Fig. 3B) and M5 (Fig. 3C). This finding suggests that the hydroxylation of M3 is similar to that of M1, and the addition of oxygen is on the *tert*-butyl group. The hydroxylation of M4 and M5 is similar to that of M2, and the hydroxyl group is on one of the ring moieties, but the exact hydroxyl position could not be determined.

The Time Course of Hydroxylated Metabolite Formation in HLMs. After incubation of 10 μM buprenorphine with HLMs, the amount of M1, M2, M3, M4, and M5 was determined by SRM of *m/z* 484 to 396 (M1), *m/z* 484 to *m/z* 101 (M2), *m/z* 471 to 202 (M3), and *m/z* 471 to 101 (M4 and M5) transitions, respectively. The amount was expressed as peak area ratio in comparison with internal standard d_4-buprenorphine because no standard compound was available. The rate of formation of M1 was greater than that of M3 and M5, as indicated by the slope of the curves at earlier incubation times. The amount of M1 decreased after 10 min, suggesting that it might undergo further metabolism (Fig. 4A). Only M3 was detected in HLMs incubated with 10 μM norbuprenorphine, and it increased linearly up to 60 min (Fig. 4B). The metabolites M2 and M4 were not detected by SRM.

Screening of 12 cDNA-Expressed Human P450s in the Metab-

nM buprenorphine (Moody et al., 2002), incubation of 10 μM buprenorphine with 12 human baculovirus insect cell-expressed P450 isoenzymes (25 pmol) showed that the 3A family and 2C8 were the major enzymes involved in buprenorphine elimination and norbuprenorphine formation (data not shown). The most efficient enzyme for M1 formation was P450 3A5, followed by 2C8, 3A4, and 3A7. The formation of M3 and M5 was mediated by P450 3A4, with a smaller contribution of 3A7 and 3A5. No metabolism was observed with other P450s and control insect microsomes (Fig. 5A). Incubation of 10 μM norbuprenorphine with P450s only produced M3, which was mainly mediated by 3A4 and, to a much lesser extent, by 3A5 (Fig. 5B).

The Contribution of Individual P450s to Buprenorphine Metabolism in HLMs. *MAb Analysis and Chemical Inhibition.* Based on our P450 screening data, together with previously reported results (Moody et al., 2002; Picard et al., 2005), P450 3A4, 3A5, 3A7, and 2C8 are the major enzymes involved in the elimination of buprenorphine. In addition, a preliminary experiment in pooled HLMs using other selective P450 inhibitors, 5 μM furafylline (1A2), 5 μM 8-methoxypsoralen (2A6), 20 μM sulfaphenazole (2C9), and 10 μM quinidine (2D6), did not show any significant inhibition on buprenorphine metabolism. Therefore, the study on the contribution of individual P450s focused on 3A4/5 and 2C8. The individual contribution of 3A4/5 and 2C8 was determined by measuring metabolite(s) formation and buprenorphine elimination in phenotyped HLMs after the addition of MAbs (see Yang et al., 1999; Krausz et al., 2001 for specificity of MAbs) or chemical inhibitors. Based on time course results, norbuprenorphine and M1 formation were evaluated at 10 min and all others at 30 min. The percentage of inhibition observed with the addition of a MAb or chemical inhibitor determined its contribution to the total metabolism (Table 1). In the current study, two phenotyped HLMs with different relative activities of 3A4/5 and 2C8 were used. HLM 452013 had higher 2C8 and lower 3A4/5 activity, whereas HLM 452164 had higher 3A4/5 and lower 2C8 activity. In HLM 452013,

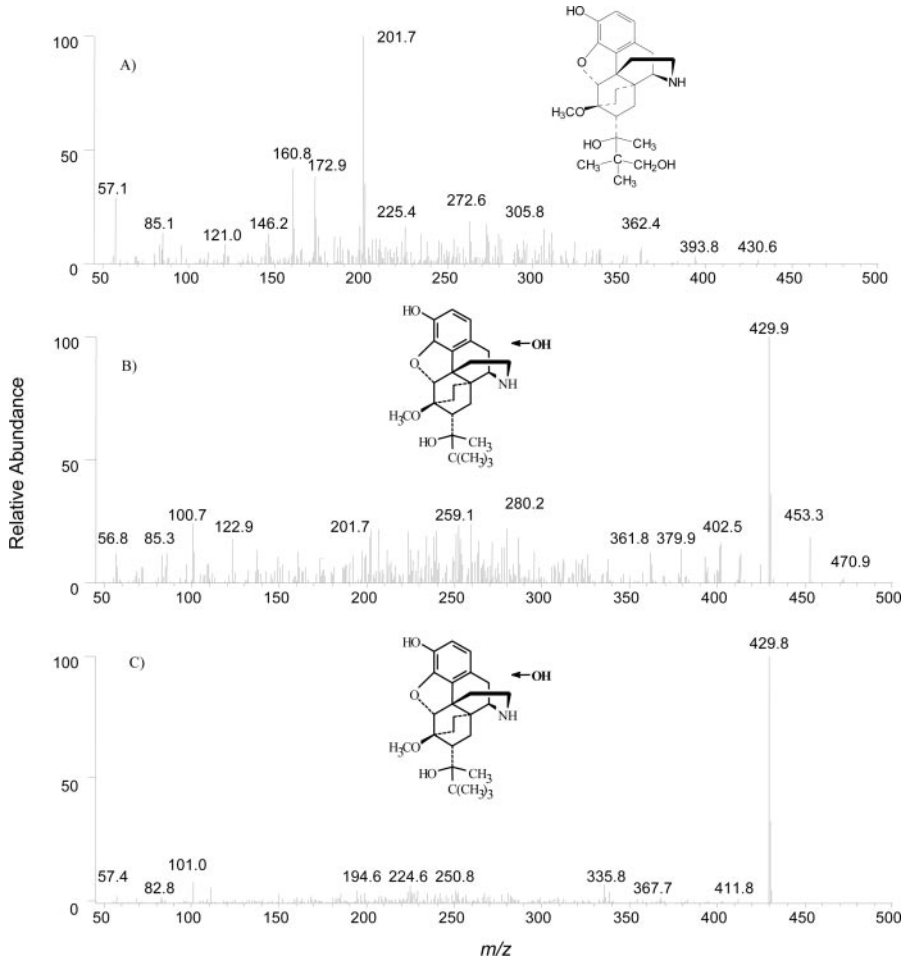

FIG. 3. CID product ion mass spectra of M3 (A), M4 (B), and M5 (C), and deduced structures.

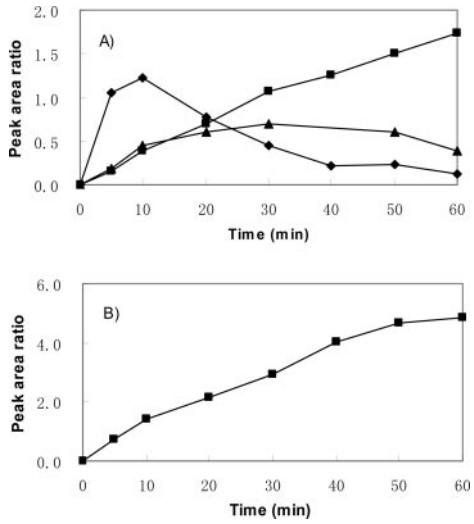

FIG. 4. The time course of M1 (♦), M3 (■), and M5 (▲) formation in HLMs incubated with 10 μM buprenorphine (A), and the time course of M3 formation in HLMs incubated with 10 μM norbuprenorphine (B). The microsomal protein content is 0.5 mg/ml. The amount of metabolites was expressed as peak area ratio of metabolites to internal standard d_4-buprenorphine. Each point is the mean of duplicate experiments.

formation of norbuprenorphine and M1 were 4.8 to 11.9 times higher than that of 3A4/5 according to MAb analysis, and 1.6 to 3.5 times same for MAb analysis and chemical inhibition, and the contribution of 3A4/5 was higher than that of 2C8. In both HLMs, the contribution of 3A4/5 was higher than that of 2C8 for the formation of M3 and M5. No significant difference was observed by increasing the amount of MAbs from 10 μl to 20 μl.

Scaling of cDNA-Expressed P450 Activities. RAFs were determined using the average of the enzyme activities for the 15 phenotyped HLMs used in this study divided by the enzyme activities provided by BD Gentest for the cDNA-expressed P450s (Crespi, 1995; Venkatakrishnan et al., 2000). The immunoquantification abundances were from another previously described (Neff and Moody, 2001) BD Gentest data bank of seven HLMs; the abundance of 2C8, which was not provided, was estimated from 2C9 abundance and the finding of Lapple et al. (2003) that the average content of 2C8 is 64.2% of 2C9. The predicted contributions of individual P450s are shown in Table 2. Using RAFs, P450 3A contributed the most to buprenorphine elimination (78.1%) and norbuprenorphine formation (48.4%), followed by 2C8, with a contribution of 14.5% and 36.4%, respectively. For the formation of M1, 2C8 was predicted to contribute most (70.2%), followed by 3A (29.2%). The estimated contribution of 3A increased and 2C8 decreased when the immunoquantitative data were used (Table 2).

Correlation Study. The rates of formation of metabolites and buprenorphine elimination were determined in 15 individual HLMs, and the data were correlated with the P450 phenotyped activities

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