MICROSOMAL CODEINE N-DEMETHYLATION: COSEGREGATION WITH CYTOCHROME P4503A4 ACTIVITY

YOSEPH CARACO,1 TOMONORI TATEISHI, F. PETER GUengerich, and ALASTAIR J. J. WOOD

Division of Clinical Pharmacology (Y.C., T.T., A.J.J.W.) and Department of Biochemistry and Center in Molecular Toxicology (F.P.G.), Vanderbilt University School of Medicine

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ABSTRACT:

Codeine is metabolized by glucuronidation, by O-demethylation to morphine, and by N-demethylation to norcodeine. The enzyme responsible for the O-demethylation to morphine has been identified as cytochrome P4502D6 (CYP2D6). The purpose of the present study was to identify the specific P450 enzyme responsible for codeine N-demethylation. Microsomal preparations (250 pmol of P450) obtained from 12 human liver donors were incubated with 20 μM codeine and analyzed for norcodeine formation. Codeine N-demethylation activity was linearly correlated with nifedipine oxidation activity (r = 0.90, p < 0.001), a marker of CYP3A4, but not with codeine O-demethylation, a marker of CYP2D6. Preincubation with troleandomycin (50 μM), or gestodene (50 μM) inhibitors of CYP3A4, decreased the rate of production of norcodeine by 60 and 45% compared to control values, respectively. Similarly, ketoconazole (10 μM) and erythromycin (10 μM) inhibited codeine N-demethylation by 75 and 35%, respectively. In contrast, the presence of quinidine, sulfaphenazole, or diethylthiocarbamate in the incubation mixture had no effect on norcodeine formation. Preincubation with antibodies raised to CYP3A4 (5 mg IgG/nmol P450) caused 96% inhibition of norcodeine production, whereas preimmune IgG or antibodies raised to CYP2A6 and CYP2C had no effect. Additionally, significant norcodeine production was observed with purified CYP3A4 derived from human liver microsomes. In conclusion, codeine N-demethylation activity cosegregates with CYP3A4 activity. Co-administration of codeine with selective inhibitors of CYP3A4 may result in increased morphine production and enhanced pharmacodynamic effects due to shunting down the CYP2D6 pathway.

Codeine is an ancient analgesic, antitussive, and anti-diarrheal drug whose therapeutic effects are principally produced by the O-demethylated metabolite, morphine (1). As with other opioids, e.g., dextro-methorphan and ethylmorphine, glucuronidation of codeine to codeine-6-glucuronide and subsequent urinary excretion is the major route of elimination (2). A third metabolic pathway which accounts for about 15% of codeine biotransformation involves N-demethylation to norcodeine (fig. 1) (2).

The formation of morphine from exogenous codeine has recently been shown to cosegregate with the polymorphic cytochrome P4502D6 (CYP2D6) enzyme (3–5). Thus, 5 to 10% of Caucasians cannot generate morphine from codeine because they are deficient in the functional enzyme (4–5). In such poor metabolizers of debrisoquin, administered codeine is ineffective in alleviating experimental pain (6). Furthermore, we have recently shown that the production of morphine from codeine in extensive metabolizers of debrisoquin is associated with a significant respiratory, psychomotor, and mictic effect. In contrast, minimal pharmacodynamic effects are seen in poor metabolizers (7). Environmental factors, in addition to genetic factors, affect the production of morphine from codeine. Inhibitors of CYP2D6 such as quinidine profoundly impair the production of morphine from codeine and markedly reduce codeine's pharmacodynamic effects (7, 8). In contrast to the O-demethylation, the enzyme responsible for codeine's N-demethylation has not been well characterized. The purpose of the present study therefore was to determine the specific cytochrome P450 responsible for the N-demethylation of codeine.

Materials and Methods

Chemicals. Codeine, norcodeine, morphine, normorphine, naloxone, diethylthiocarbamate, ketoconazole, troleandomycin, and erythromycin were purchased from Sigma Chemical Co. (St. Louis, MO). Quinidine and α-naphthoflavone were obtained from Aldrich Chemical Co. (Milwaukee, WI). Gestodene was provided by Professor H. Kuhl (University of Frankfurt) and sulfaphenazole was a gift from the Meiji Yakuhim Co. Ltd. (Tokyo, Japan). All other chemicals were obtained from various commercial sources and were of the highest analytical grade.

Liver Microsomes and Incubation Conditions. Liver samples were obtained from 12 different liver donors through Tennessee Donor Services (Nashville, TN). Microsomes were prepared as described elsewhere (9) and the P450 content was determined by Fe2+-CO versus Fe2+ difference spectroscopy (10). Incubation conditions were determined from preliminary studies that demonstrated linear formation of norcodeine at incubation times ranging from 20 to 60 min, P450 content ranging from 50 to 250 pmol per incubation vial, and codeine concentration ranging from 4 to 50 μM. Thus, a mixture of 20 μM codeine, 170 mM Tris-HCl (pH 7.4), 5 mM MgCl2, and 250 pmol microsomal protein/ml was preincubated (in a final volume of 1 ml) for 5 min in open glass vials immersed in a shaking bath at a constant temperature of 37°C. The reaction was initiated by the addition of NADPH-generating system consisting of 2.5 mM glucose 6-phosphate, 0.5 mM NADP+, and one IU of glucose 6-phosphate dehydrogenase/ml. The reaction was terminated 45 min later by the addition of 1 ml of 1 M sodium bicarbonate buffer (pH 10) to the incubation vials, which were then immediately immersed in ice.

Assays. Measurement of codeine, norcodeine, morphine, and normorphine

Send reprint requests to: Alastair J. J. Wood, M.D., Division of Clinical Pharmacology, Vanderbilt University School of Medicine, 552 MRB Building, Nashville, TN 37232-8602.
from the microsomal incubation mixtures was performed using modifications of a previously described HPLC method (11). Following termination of the microsomal reaction, 50 μl of the internal standard, naloxone (1 μg), and 8 ml of 15% (v/v) propanol/dichloromethane were added to each incubation vial. The mixture was then mixed for 10 min and centrifuged for 5 min at 1000 rpm in a benchtop centrifuge. The organic phase (7.5 ml) was removed to another tube and the solvent was evaporated under a gentle stream of N₂ at 37°C. The residue was then reconstituted with 100 μl mobile phase (see below) and 40 μl was injected onto a 4.6 mm × 150 mm ultrashare ODS 5 μm column (Beckman, San Ramon, CA) fitted with a reversed phase guard column (Whatman, Piscataway, NJ). The mobile phase consisted of 12.5% (v/v) acetonitrile in 20 mM phosphate buffer pH 6.65. Under these chromatographic conditions the retention times of normorphine, morphine, norcodeine, codeine, and naloxone were 3, 5.1, 7.1, 14.7, and 30 min, respectively. Nifedipine oxidation activity was determined in each microsomal preparation, as previously described (12).

Chemical Inhibition. The effect of increasing concentrations of known specific cytochrome P450 inhibitors such as gestodene (CYP3A4; 10, 20, 30, and 50 μM), troleandomycin (CYP3A4; 10, 20, 30, and 50 μM), erythromycin (CYP3A4; 2.5, 5, and 10 μM), diethyldithiocarbamate (CYP2E1; 10, 20, and 30 μM), sulfaphenazole (CYP2C9 and CYP2C10; 10, 20, and 30 μM), ketoconazole (2, 5, and 10 μM), and quinidine (CYP2D6; 1, 2, and 5 μM) (13) on the formation rate of norcodeine in microsomal preparations obtained from a single donor were examined under the same incubation conditions as described above. Gestodene, troleandomycin, and diethyldithiocarbamate were preincubated with microsomes and the NADPH-generating system at 37°C for 20 min before adding codeine (14). The effects of increasing concentrations of α-naphthoflavone, a known CYP3A4 activator (10–100 μM) (15) were also determined under similar conditions.

Immunoinhibition. Immunoinhibition was examined by preincubating microsomes obtained from a single donor with increasing concentrations (1, 2, 5, and 10 mg IgG/nmol P450) of preimmune globulin or specific antibodies raised to CYP2A6, CYP2C, and CYP3A4 for 20 min at 23°C. Codeine (20 μM) and the components of the NADPH-generating system were then added and the reaction was allowed to continue for an additional 45 min as described above.

Purified Cytochrome P450 Systems. Recombinant CYP1A2 (16), CYP2C9/10 (17), and CYP3A4 (18) (100 pmol) were reconstituted with human cytochrome b₅ (19) (15 pmol), 200 pmol of rabbit NADPH cytochrome P450 reductase (20–22), and 30 μM diatursyphosphatidylcholine in 170 mM Tris-HCl buffer (pH 7.4) (22). The production of norcodeine by these reconstituted enzymes was evaluated following incubation with codeine (20 μM) and NADPH-generating system at 37°C for 45 min.

Statistical Analysis. The association between the formation rate of norcodeine, morphine, and nifedipine oxidation activity was examined by linear regression analysis. A p value of less than 0.05 was considered statistically significant.

Results

Measurable amounts of norcodeine were formed by all 12 human liver microsomal preparations. An almost 8-fold variation in the formation rate of norcodeine from codeine was noted among the different microsomal preparations ranging from 5.2 to 40.2 (pmol norcodeine formed/min/nmol P450). Norcodeine production rates were significantly correlated with nifedipine oxidation activity, a marker of CYP3A4 activity (r = 0.90, p < 0.001; fig. 2) but not with codeine O-demethylation to morphine, a CYP2D6-mediated pathway (r = 0.14, p > 0.5; fig. 3).
Preincubation with troleandomycin or gestodene produced marked inhibition of norcodeine production (fig. 4). At the highest concentrations of troleandomycin and gestodene (50 μM), norcodeine production was inhibited by 60 and 45%, respectively. Ketoconazole also significantly inhibited norcodeine formation; at 10 μM norcodeine production rate was decreased by approximately 75% (fig. 5). Erythromycin was a less potent inhibitor; at 10 μM it inhibited codeine N-demethylation by only 35% (fig. 5). Quinidine, sulfaphenazole, diethylthiocarbamate, and α-naphthoflavone had no effect on norcodeine formation rate (fig. 6).

Preincubation of microsomes from a single donor with specific antibodies (2.5 mg of IgG/nmol of P450) (12) raised to CYP3A4 decreased codeine N-demethylation activity by 65% (fig. 7). At higher concentrations of antibody norcodeine production was essentially abolished (fig. 7). Preimmune IgG or specific antibodies raised to CYP2A6 or CYP2C had no effect on the formation rate of norcodeine (fig. 7). These antibody studies demonstrate that all of the norcodeine production is mediated by CYP3A (and hence inhibited by antibody to it). The lack of complete inhibition by chemical inhibitors presumably reflects the different affinities of the compound and substrate for CYP3A.

Purified human CYP3A4 formed norcodeine from codeine at the rate of 8.8 pmol/min/nmol P450 consonant with the microsomal results; in contrast, norcodeine was not formed following codeine incubation of reconstituted CYP1A2 or CYP2C.

Discussion

Codeine biotransformation takes place principally by glucuronidation to codeine 6-glucuronide, which is then eliminated through renal excretion (2). O-Demethylation of codeine accounts for less than 10% of codeine metabolism and is mediated by CYP2D6 (3–5). In the present study we have shown that CYP3A4 is the major enzyme responsible for the formation of norcodeine through codeine N-demethylation.

CYP3A4 accounts for more than 20% of cytochrome P450 in human liver (14) and the level can be as high as 60%. This enzyme metabolizes a large number of drugs including antiarrhythmics such as quinidine and lidocaine, antibiotics such as dapsone, erythromycin, and troleandomycin, immunosuppressants such as cyclosporine and FK506, and benzodiazepines such as midazolam and triazolam (23). Our finding that N-demethylation of codeine is catalyzed by CYP3A4 is consonant with previous studies of two structurally related opioids, ethylmorphine and dextromethorphan, whose N-demethylation also correlates with CYP3A4 activity (24–25).
The recognition of CYP3A4 as the enzyme responsible for the N-demethylation of codeine may be of clinical importance both because of the marked intersubject variation in this enzyme activity (26–27) and because inhibition of CYP3A4 may result in more of codeine’s metabolism being shunted down the CYP2D6 pathway to morphine with a resultant increase in codeine’s pharmacodynamic effects. In support of this suggestion we have recently shown that rifampin treatment for 3 weeks markedly enhanced codeine N-demethylation with a significant reduction in morphine plasma concentrations and a reduction in codeine’s pupillary, respiratory, and psychomotor effects (28). Our identification of CYP3A4 as the enzyme responsible for codeine N-demethylation and the recognition that rifampin is a potent CYP3A4 inducer (29) further illustrates how the use of an in vitro technique can explain and predict clinically significant drug interactions.

References


