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BIOACTIVATION OF THE NARCOTIC DRUG CODEINE IN HUMAN LIVER IS MEDIATED BY THE POLYMORPHIC MONOOXYGENASE CATALYZING DEBRISOQUINE 4-HYDROXYLATION (cytochrome P-450 db1/bufI)

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Codeine O-demethylation to its active moiety morphine was investigated in human liver microsomes from 1 poor and 5 extensive metabolizer subjects (debrisoquine-type of oxidation polymorphism). Apparent Km of the reaction in one extensive metabolizer's microsomes was 149 μM and Vmax 17.6 nmol x mg P^{-1} x $hour^{-1}$ versus > 1 mM and 1.6 nmol x mg P^{-1} x $hour^{-1}$ respectively in one poor metabolizer. In vitro morphine production was competitively inhibited by quinidine (Ki 15 nM), the selective inhibitor of cytochrome P-450 db1/bufI. There was also an excellent correlation between dextromethorphan O-demethylation, a prototype reaction for cytochrome P-450 db1/bufI activity, and codeine O-demethylation. These data allow to conclude that codeine bioactivation to morphine is dependent on the polymorphic monooxygenase known as cytochrome db1/bufI.

The intensity and duration of effect of many drugs depend on the activity of drug metabolizing enzymes in the liver. Of primary importance among them are the microsomal polysubstrate monooxygenases (cytochrome P-450 isozymes). Efforts to elucidate the mechanisms underlying differences in cytochrome P-450 function have been hampered by the extreme difficulty of separating the influence of each isozyme on a defined metabolic reaction, due to the overlapping substrate specificity of these isozymes. The recent identification of genetic polymorphisms of drug oxidation related to cytochrome P-450 function has provided tools to discover more specific substrates for given isozymes (1,2). The best studied example is the debrisoquine-type of polymorphism (1,3-9), which is due to a deficiency in cytochrome P-450 db1/bufI (6-9), homologue of the rat cytochrome P-450 db1 (9,10). About 10 % of Caucasians are homozygous for an autosomal recessive gene (poor metabolizers) (3) and have impaired capacity to metabolize

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debrisoquine, sparteine, bufuralol, dextromethorphan and more than 30 other drugs (11,12). The clinical relevance of this polymorphism has been demonstrated, poor metabolizer subjects having a propensity to develop adverse drug reactions (11,12).

In human liver microsomes, (+)-bufuralol 1'-hydroxylation (6-8) and dextromethorphan O-demethylation (13,14) are two prototype reactions for the assessment of cytochrome P-450 dbl/bufI function that have shown predictive value for in vivo behavior (15). Both reactions allow for in vitro screening of candidate substrates (competitive inhibitors) for the polymorphic isozyme. We found that codeine and other narcotics competitively inhibit bufuralol and dextromethorphan oxidations in human liver microsomes. Codeine O-demethylation to its active moiety morphine was therefore investigated in liver microsomal preparations from organ transplantation donors.

MATERIALS AND METHODS

Liver tissue: The experimental protocol was approved by the ethical review board (Department of Medicine, University of Geneva). Human liver samples were obtained from organ transplantation donors and stored as described (5). Using in vitro function assays (7, 13, 14), 5 of the studied samples were classified as "extensive metabolizers" (debrisoquine-type phenotype) and one as "poor metabolizer". However, as these tests for cytochrome P-450 dbl activity are appropriate for extensive metabolizer phenotyping only, the phenotype of the poor metabolizer (liver #03) was confirmed by immunoquantitation of cytochrome P-450 dbl/bufI content in microsomal membranes. Intensity of the Western immunoblot dbl band using a specific antibody , measured by TLC scanning in reflection mode, was that of a typical poor metabolizer (9).

Enzymatic assays in microsomes: Human liver microsomes were prepared, and bufuralol 1'-hydroxylation or dextromethorphan O-demethylation were assayed as previously described (7,13). For the codeine O-demethylation assay, 200 µg of microsomal protein (16) (ca. 5o pmoles of total cytochrome P-450) were incubated in a final volume of 200 µl of 0.1 M NaPO4 containing a NADPH regenerating system (1 mM NADP, 5 mM isocitrate, 5 mM MgCl2 and 1 unit of isocitrate dehydrogenase-type IV -SIGMA, St. Louis, MO, USA). The system containing codeine phosphate at final concentrations ranging from 5 to 600 µM was preincubated at 37 °C for 5 min before addition of microsomes. For inhibition experiments, quinidine sulfate dissolved in 0.1 M NaPO4 was added at the same time as substrate, at final concentrations ranging from 10 nM to 1 µM. After 30 min the reaction was stopped on ice, pindolol was added as internal standard and incubates were poured on a Supelclean LC-18 column (SUPELCO, Bellefonte, PA, USA). After evaporation of the column eluate, the production of morphine was determined by HPLC. Aliquots (50 µl) of redisolved extract (0.1 M phosphate buffer) were injected on a 5 µm LC-DP column (SUPELCO). The mobile phase was 10 mM phosphate buffer, pH3 - acetonitrile (80/20, v/v) and triethylamine (100 µl/1). Column effluent was monitored with

a fluorescence detector (Schoeffel FS 970, Kratos Inc., Westwood, NJ, USA) at an excitation/emission wavelength pair of 210/360-380 nm.

Data analysis and statistics: Untransformed kinetic data were analyzed by means of a non-linear extended least squares curve fitting program which allows an observer-independent weighting of the data (17). The data from the kinetic study were treated according to Michaelis-Menten type kinetics, which offered adequate description of the results in the substrate range studied. For correlation between codeine and dextromethorphan, and for group comparisons, non-parametric tests were used (Spearman rank correlation, Mann-Whitney and Sign-tests) (18).

RESULTS AND DISCUSSION

Morphine production from codeine, in extensive metabolizer's and in poor metabolizer's microsomes, is shown in figure 1. Apparent Michaelis constants are very different: 149 μM in extensive (liver #20) versus more than 1000 μM in poor metabolizers' microsomes (liver #03). Such a difference in Km values is very similar to that previously observed with the prototype compounds (+)-bufuralol and dextromethorphan (7,13). In extensive metabolizers' microsomes, like previously shown for bufuralol, the low Km results from a mixed contribution of two isozymes, showing high and low affinity respectively (7, 8). In poor metabolizers' microsomes, loss of the high affinity component results in a shift to a higher apparent Km (7). The phenotypes also differ in the rate of morphine production: Vmax in extensive metabolizer's microsomes is 17.6 nmol x mg P^{-1} x $hour^{-1}$ versus 1.6 nmol x mg P^{-1} x $hour^{-1}$ for poor metabolizer's microsomes.

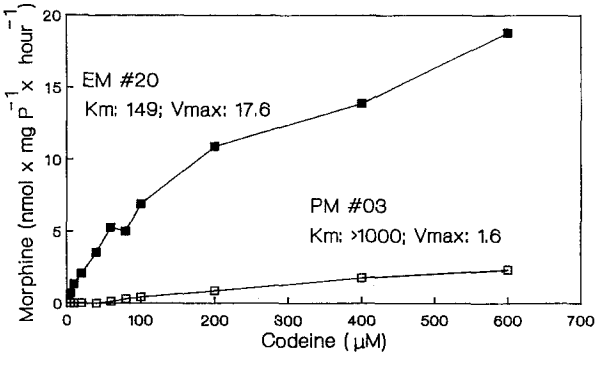

Figure 1: Kinetics of morphine production from codeine in one extensive metabolizer's (EM) and one poor metabolizer's (PM) microsomal preparation. Each point is the mean of 2 measures.

Table I: Production rate (nmol x mg P^{-1} x $hour^{-1}$) of morphine from codeine (500 μM), and dextrorphan from dextromethorphan (20 μM), in 6 human liver microsomal preparations (rho_S = 1.00, $p<0.01$)

Liver	(phenotype)	Morphine	Dextrorphan
#02	(EM)	6.66	4.86
#04	(EM)	4.40	3.60
#20	(EM)	19.54	12.00
#25	(EM)	7.55	7.64
#26	(EM)	20.87	14.00
#03	(PM)	1.84	0.58

Each value is the mean of 2 measures; EM = extensive metabolizer's and PM = poor metabolizer's microsomes.

Table I shows the correlation between morphine and dextrorphan (= O-demethylated dextromethorphan) productions, under Vmax conditions, in the 6 livers. The concordance between dextromethorphan, a prototype substrate for evaluation of cytochrome dbl/bufI function, and codeine oxidations is excellent, suggesting that codeine O-demethylation is mediated by the same polymorphic monooxygenase.

Direct evidence that O-demethylation of codeine to morphine is mediated by the polymorphic monooxygenase would require measurement in a reconstituted system containing purified cytochrome P-450 dbl/bufI. However, strong indirect evidence can be obtained from experiments with selective inhibitors. At low concentration, quinidine selectively and competitively inhibits the polymorphic monooxygenase (7,19). The Ki of quinidine for the high affinity/stereoselectivity component of the prototype reaction (+)-bufuralol 1'-hydroxylation in 5 different liver microsomal preprarations ranged from 3 to 13 nM (7). The quinidine Ki for codeine O-demethylation is 15 nM (liver #20), again confirming that both reactions are mediated by the same isozyme. Figure 2 shows the influence of quinidine on morphine production in extensive and poor metabolizers' microsomes. The mean output of morphine in 5 extensive metabolizers' microsomes was 11.80 (SD 3.47) in the absence and 2.58 (0.60) nmol x mg P^{-1} x $hour^{-1}$ in the presence of 1 μM quinidine ($p<0.05$), whereas the poor metabolizer's microsomes produced 1.84 and 1.80 nmol x mg P^{-1} x $hour^{-1}$ in these conditions.

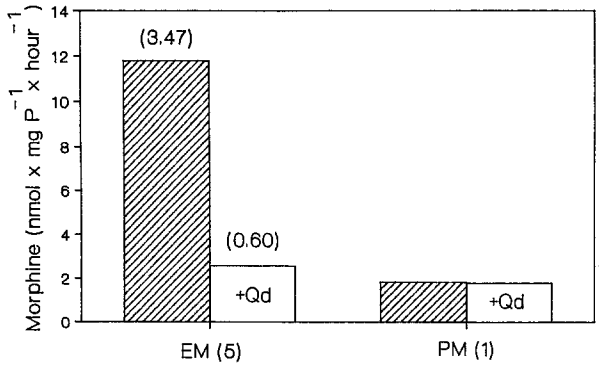

Figure 2: In vitro morphine production from codeine (500 µM), in the absence or presence of 1 µM quinidine (+Qd), in 5 extensive metabolizers' (SD) and 1 poor metabolizer's microsomal preparations.

We conclude that the rate of codeine bioactivation to morphine is controlled by the activity of the polymorphic cytochrome P-450 dbl/bufI. The clinical relevance of this observation is clear when it is recalled that about 10 % of Caucasians lack this metabolic pathway. Poor metabolizer subjects cannot activate this widely used drug, and for them codeine is an ineffective analgesic. Moreover, due to the high Km of codeine for the polymorphic isozyme, its bioactivation in extensive metabolizer subjects is liable to vary greatly when it is combined with any drug that has a high affinity for the enzyme, for example antiarrhythmic agents or antidepressants (7, 20). Lastly, the observation that biotransformation, and thus intensity and duration of effect, of a narcotic drug may be genetically determined opens a new field in addiction research.

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