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STERIC CONFIGURATION AND POLYMORPHIC OXIDATION OF LIPOPHILIC BETA-ADRENOCEPTOR BLOCKING AGENTS: IN VIVO - IN VITRO CORRELATIONS

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The oxidation polymorphism (debrisoquine-type) has been identified as one of the major factors responsible for interindividual variations in the fate of certain lipophilic beta-blockers such as bufuralol (1-3) and metoprolol (4). Poor metabolizers (PM), who make up slightly less than 10 % of the population in European countries, have higher plasma concentrations of unchanged drug (1-4) and sometimes are at higher risk for adverse side-effects (2). Most beta-blockers are used in medicine as a mixture of two enantiomorphic isomers of different potencies and many monooxygenase reactions are characterized by stereospecific recognition of substrates with asymmetric carbon centers such as beta-blockers (5,6). Therefore, using bufuralol and metoprolol as test compounds, the influence of substrate stereoselectivity for the genetically controlled metabolic pathway was studied in vivo and in vitro.

Subjects and Methods

(a) in vivo study : selected extensive (EM, n=6) and poor (PM, n=4) metabolizers for debrisoquine received racemic bufuralol (30 mg) and metoprolol (100 mg) orally. Plasma samples were collected 3 h after drug administration. The parent drug concentrations were determined by means of chiral liquid chromatography (7) which allows direct measurements of each enantiomer. In other trials racemic, (+)- and (-)-bufuralol (2.5 mg) were administered orally to selected EM (n=2) and PM (n=2). Bufuralol, 1'hydroxy-, 1'keto-, 4-hydroxy-, 6-hydroxy- and conjugated-bufuralol were assessed in 8 h urine collections by means of a GC-MS assay (8).

(b) in vitro study : human microsomes were prepared from wedge liver biopsies of previously phenotyped subjects or whole livers from kidney donors (9). (+)- and (-)-bufuralol oxidations to 1'-hydroxy-bufuralol were monitored by means of a HPLC assay. Incubations were performed at 37°C in 0.1 mol/L $NaPO_4$ buffer pH 7.4, containing a NADPH regenerating system and 50 µg microsomal protein in a volume of 250 µl (10). In other trials the oxidation kinetics were monitored in a non-membraneous reconstituted system containing a purified human cytochrome P-450 isozyme with a high activity for bufuralol oxidation, NADPH-cytochrome P-450 reductase, phospholipids and a NADPH regenerating system (10).

Results and Comments

In plasma, bufuralol (-)/(+) ratios are 1.60 ± SD 0.25 in EM versus 2.65 ± 0.24 in PM. The increase in the ratio in PM is due to a stereoselective glucuronidation and elimination of (+)-bufuralol not observed with metoprolol. Bufuralol 1'-hydroxylation is under genetic control and selective for the (+)-isomer. Bufuralol 4- and 6-hydroxylations (aromatic oxidations) are under the same genetic control but selective for the (-)-isomer. The selectivity of both types of oxidations is virtually abolished in PM. Metoprolol oxidation is also under genetic control and stereoselective. Plasma metoprolol (-)/(+) ratios are 1.85 ± 0.29 in EM versus 1.07 ± 0.11 in PM. In EM microsomes the aliphatic hydroxylation of bufuralol to 1'-hydroxybufuralol displays simple Michaelis-Menten kinetics. The reaction is also selective for the (+)-isomer (Figure 1) with a (-)/(+) ratio ranging from 0.3 to 0.6 depending on the liver tested. In PM microsomes not only the total metabolite formation is reduced but the substrate selectivity is almost completely suppressed with a (-)/(+) ratio ranging from 0.7 to 0.9. In the non-membraneous system containing the purified isozyme the 1'-hydroxylation shows a marked increase in stereoselectivity with a mean (-)/(+) ratio of 0.13 (0.11-0.15) (10).

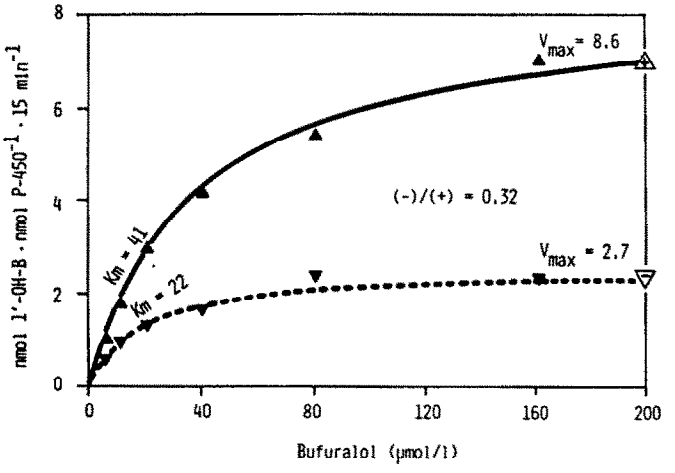

Figure 1 : bufuralol 1'-hydroxylation in EM microsomes.

The stereoselective oxidations observed in EM are in agreement with previously published work (5,6,9). The polymorphic metabolism involves the aliphatic and the aromatic hydroxylations of bufuralol and these reactions display an opposite stereoselectivity. The poor metabolizers are characterized by a loss of the stereoselective component *in vivo* and *in vitro*. The marked increase in stereoselectivity observed with the purified isozyme suggests strongly that this protein is the major "bufuralol-debrisoquine" hydroxylating species and the target of the genetic deficiency.

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