Involvement of Human CYP1A Isoenzymes in the Metabolism and Drug Interactions of Riluzole In Vitro

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ABSTRACT

Cytochrome P450 (CYP) and uridine diphosphate glucuronosyltransferase (UGT) isoenzymes involved in riluzole oxidation and glucuronidation were characterized in (1) kinetic studies with human hepatic microsomes and isoenzyme-selective probes and (2) metabolic studies with genetically expressed human CYP isoenzymes from transfected B-lymphoblastoid and yeast cells. In vitro incubation of [14C]riluzole (15 μM) with human hepatic microsomes and NADPH or UDPGA cofactors resulted in formation of N-hydroxyriluzole (Km = 30 μM) or an unidentified glucuroconjugate (Km = 118 μM). Human microsomal riluzole N-hydroxylation was most strongly inhibited by the CYP1A2 inhibitor α-naphthoflavone (IC50 = 0.42 μM). Human CYP1A2-expressing yeast microsomes generated N-hydroxyriluzole, whereas human CYP1A1-expressing yeast microsomes generated N-hydroxyriluzole, two additional hydroxylated derivatives and an O-dealkylated derivative. CYP1A2 was the only genetically expressed human P450 isoenzyme in B-lymphoblastoid microsomes to metabolize riluzole. Riluzole glucuronidation was inhibited most potently by propofol, a substrate for the human hepatic UGT HP4 (UGT1.8/9) isoenzyme. In vitro, human hepatic microsomal hydroxylation of riluzole (15 μM) was weakly inhibited by amitriptyline, diclofenac, diazepam, nergicline, clomipramine, imipramine, quinine and enoxacin (IC50 = 200–500 μM) and cimetidine (IC50 = 940 μM). Riluzole (1 and 10 μM) produced a weak, concentration-dependent inhibition of CYP1A2 activity and showed competitive inhibition of methoxyresorufin O-demethylase. Thus, riluzole is predominantly metabolized by CYP1A2 in human hepatic microsomes to N-hydroxyriluzole; extrahepatic CYP1A1 can also be responsible for the formation of several other metabolites. Direct glucuronidation is a relatively minor metabolic route. In vivo, riluzole is unlikely to exhibit significant pharmacokinetic drug interaction with coadministered drugs that undergo phase I metabolism.

Riluzole1 [2-amino-6-(trifluoromethoxy)benzothiazole], a novel antiglutamate agent with neuroprotective properties in animal models of neurodegenerative disease (Doble, 1996), has been shown to prolong survival in patients with ALS (Bensimon et al., 1994; Lacomblez et al., 1996). After oral administration to humans, the drug is almost completely absorbed, undergoes limited first-pass metabolism and is excreted predominantly via the urine in the form of metabolites resulting from phase I and II metabolism.2,3

Characterization of the CYP isoenzymes responsible for the metabolism of riluzole is of importance in assessing the likelihood of pharmacokinetic variability due to genetic polymorphism and differential regulation and in identifying potential drug interactions. In the present study, the in vitro oxidative metabolism and glucuronidation of riluzole were investigated using human hepatic microsomes. Identity of the CYP isoenzymes involved in riluzole biotransformation was established using genetically expressed human CYP isoenzymes from transfected cell lines and yeast and isoenzyme-selective inhibitory probes. Similarly, pathways of hepatic microsomal glucuronidation of riluzole were investigated with known inhibitors/substrates of UGT isoenzymes. To identify potential metabolic drug interactions, the effects of known CYP substrates/inhibitors and frequently coadministered drugs on the hepatic microsomal oxidation of riluzole and, conversely, the effects of riluzole on specific human hepatic CYP-dependent drug metabolism reactions were determined.

Materials and Methods

Chemicals

Riluzole, N-hydroxyriluzole (RPR 112512), 4-hydroxyriluzole (RP 65077), 5-hydroxyriluzole (RP 65110), 7-hydroxyriluzole (RP 65331)

ABBREVIATIONS: ALS, amyotrophic lateral sclerosis; CYP, cytochrome P450; NADPH, reduced nicotinamide adenine dinucleotide phosphate; UGT, uridine diphosphate glucuronosyltransferase; UDPGA, uridine diphosphate glucuronic acid.
and 2-amino-6-hydroxybenzothiazole (RPR 109792) were synthesized at the Centre de Recherche de Vitry/Alfortville, Rhône-Poulec Rorer (France) and the Collegeville Chemical Processing Center, Rhône-Poulec Rorer (Collegeville, PA). [14C]Riluzole (radiochemical purity, >99%, specific activity, 56 mCi/mmol) was synthesized by the Service of Labeled Molecules of the Commissariat à l’Énergie Atomique (Gif-sur-Yvette, France). Aspirin, captopril, diazepam, enoxacin, imipramine, metronidazole, nicergoline, paracetamol, pefloxacain, ranitidine and sparfloxacin were obtained from Rhône-Poulec Rorer. Baclofen, amitriptyline, amoxicillin, chlorpropanide, chlorozazone, cimetidine, clonazepam, coumarin, methoxyresorufin, nifedipine, resorufin, theophylline, thiamine, tobutamide, trolen-domycin, anilin, isoniazid, α-naphthoflavone, reduced NADPH, UDPGA, sulfaphenazole, Brij-58, 1-naphthol, 4-methylumbelliferone, lithocholic acid, bilirubin, androsterone and β-estradiol were purchased from Sigma Chemical Co. (St. Louis, MO). Furafylline and S-mephenytoin were purchased from Ultrafine Chemicals (Manchester, UK); acetaldehyde and quinidine sulfate were obtained from E. Merck (Darmstadt, Germany); caffeine and quinine sulfate were from Prolabo (Paris, France); SR-mephenytoin was from Sandoz (Basel, Switzerland); ketoconazole was from Biomol Research Laboratories (Plymouth Meeting, PA) and bufuralol was from Gentest Corp. (Woburn, MA). All other reagents were obtained from commercial sources and were of analytical grade.

**Biological Materials**

**Human liver samples and preparation of microsomes.** Human liver samples were obtained from male and female organ transplant donors (Eurotransplant) or from surgery (Hôpital Cochin, Paris, France). Microsomal fractions were prepared by differential ultracentrifugation. After tissue homogenization in 20 mM Tris-HCl buffer, pH 7.4, containing NADPH (1 mM) and MgCl₂ (10 mM), microsomes were performed with a suspension of hepatic microsomes. For oxidative reactions, incubations with labeled and unlabeled drug in the presence of the respective cofactors and NADPH and UDPGA. For oxidative reactions, incubations with hepatic microsomes were performed with a suspension of hepatic microsomes (protein content, 2 mg/ml) in potassium phosphate buffer (0.1 M, pH 7.4) containing NADPH (1 mM) and MgCl₂ (10 mM). Lymphoblast B-cell microsomes were incubated in the same medium at a 0.5 mg/ml protein content, according to the supplier’s instructions, resulting in final enzyme concentrations of 8, 18, 105, 38 and 17 pmol/mg for CYP1A1, CYP1A2, CYP2D6, CYP2E1 and CYP3A4, respectively. Yeast microsomes were incubated in Tris-HCl buffer (50 mM, pH 7.4) containing EDTA (1 mM) and NADPH (1 mM) in the absence of cytochrome b₅. For yeast microsome incubations, the final CYP450 content was 200 pmol/mg. For glucuronidation reactions, [14C]Riluzole (15 µM) was incubated with a suspension of hepatic microsomes (protein content, 1 mg/ml) in potassium phosphate buffer (0.1 M, pH 7.4) containing UDPGA (5 mM) and MgCl₂ (5 mM). For glucuronidation reactions, microsomes were activated with an optimal concentration (0.2 mg/mg of protein) of Brij-58 detergent. Incubations were performed at 25°C (yeast microsomes) or 37°C (hepatic and B-cell microsomes) in an agitating water bath, and reactions were initiated by the addition of NADPH (oxidative metabolism) or UDPGA (glucuronidation). Riluzole incubation mixtures were sampled until 20 min for monooxygenase-catalyzed reactions (30 min for expressed enzymes) and until 60 min for glucuronosyltransferase-catalyzed reactions. Reactions were terminated by the addition of an equivalent volume of methanol/acetone (3:6:1 v/v) to the incubation mixture. The resulting mixture was then centrifuged at 30,000 × g for 10 min, and the supernatant was stored at 4°C for analysis.

**Enzyme kinetics.** Enzyme kinetic studies were performed by incubation of [14C]Riluzole at concentrations of 2 to 1000 µM with hepatic and CYP1A2-expressing yeast cell microsomes.

**Interaction studies.** In inhibition studies, hepatic microsomes were preincubated for 10 min with varying concentrations of CYP isoenzyme substrates/inhibitors (1–1000 µM) or UGT inhibitors (1–100 µM) in the presence of the appropriate cofactor (NADPH or UDPGA) before the addition of [14C]Riluzole (15 µM). Specific CYP isoenzyme probes included α-naphthoflavone, acetaldehyde and caffeine for CYP1A (Birkett et al., 1993; Gonzalez, 1992), tolbutamide and sulfaphenazole for CYP2C8/9 (Birkett et al., 1993), omeprazole and mephenytoin for CYP2C19 (Andersson et al., 1993; Birkett et al., 1993), quinidine for CYP2D6, with quinine as a negative control (Birkett et al., 1993; Gonzalez, 1992), aniline, p-nitrophenol, chlorozazone and isoniazid for CYP2E1 (Birkett et al., 1993; Zand et al., 1993) and ketoconazole and trolen-domycin for CYP3A4 (Back et al., 1989; Birkett et al., 1993). UGT inhibitors included propofol, 1-naphthol, 4-methylumbelliferone, lithocholic acid, bilirubin, androsterone, estradiol and p-nitrophenol.

**Metabolic drug/drug interactions.** In drug interaction studies with riluzole used as the substrate, hepatic microsomes were preincubated for 10 min with clomipramine, diclofenac, amitriptyline, imipramine, enoxacin, quinine, theophylline, cimetidine, caffeine, ranitidine, paracetamol, pyridoxine, enalapril, thiamine, captopril, pefloxacain, aspirin, amoxicillin, metronidazole, piracetam, baclofen, nicergoline or diazepam at concentrations ranging from 2 to 1000 µM in the presence of NADPH before the addition of [14C]Riluzole (15 µM). Incubations were carried out in triplicate for experiments with single inhibitor concentrations; for determination of IC₅₀ values, single incubations were performed at multiple inhibitor concentrations. Kᵢ determinations were performed in duplicate.

**Effects of riluzole on marker enzyme activities.** In an additional series of drug interaction studies, the inhibitory effects of riluzole were determined on the following CYP-selective oxidative reactions: nifedipine dehydrogenation (a marker for CYP3A4) (Guengerich et al., 1986); chlorozoxazone-6-hydroxylation (a marker for CYP2E1) (Peter et al., 1991); bufuralol-1-hydroxylation (a marker for CYP2D6) (Kronbach et al., 1987); S-mephenytoin-4-hydroxylation (a marker for CYP2C19) (Meier et al., 1985; Wrighton et al., 1993); tolbutamide-4-hydroxylation (a marker for CYP2C9) (Knodell et al., 1987; Veronese et al., 1991); coumarin-7-hydroxylation (a marker for CYP2A6) (Pearce et al., 1992; Yun et al., 1991); and phenacetin O-deethylation (a marker for CYP1A2) (Disterath et al., 1985; Sattler et al., 1992). Human hepatic microsomes were incubated with riluzole (1 and 10 µM), and the various enzyme substrates at concentrations approximating or in excess of published Kₘ values. Parallel experiments were conducted with two human liver samples, and for each assay, analyses were performed in duplicate or triplicate with a NADPH- or glucose-6-phosphate-free control to quantify non-enzymatic drug metabolism. All incubation mixtures were analyzed by high-performance liquid chromatography.
The kinetic methoxyresorufin O-demethylation assay was performed on 96-well microplates in 200 μl of potassium phosphate buffer (75 mM, pH 7.64), 9 mM KCl and 1.4 mM NADH at 37°C with 0.2 mg/ml human liver microsomes or 6.6 pmol/ml yeast-expressed CYP1A2. Methoxyresorufin dissolved in DMSO and riluzole dissolved in methanol were added together to the incubation mixture, and the reaction was initiated by the addition of 500 μM NADPH (final concentration). The final incubation mixture contained 0.5% (v/v) of both solvents. Methoxyresorufin concentrations were 0.1, 0.3, 0.5, 2 and 5 μM. For each methoxyresorufin concentration, riluzole concentrations of 0, 3, 15, 30, 150 and 300 μM were tested. Resorufin production was monitored continuously with fluorescence detection (excitation wavelength, 544 nm; emission wavelength, 590 nm) over 10 min using a LabSystems Fluoroscan II microplate spectrofluorometer controlled by Biolise software. Concentrations were calculated from a resorufin standard curve.

Sample Analysis

Analysis of riluzole metabolites was carried out by high-performance liquid chromatography with a Kontron 360 automatic sampler, a 420 solvent delivery pump, a Kontron 430 UV detector (265 nm) and a Berthold LB507A radiodetector equipped with a 500-μl flow cell. The system was controlled by a Kontron MT2 Datasystem. Separation was achieved on a Lichrocart 125 × 4-mm guard column, both packed with Lichrosphere 60 RP Select B 5-μm particles (Merck Clevenot). The mobile phase consisted of 10 mM K2HPO4/methanol/acetonitrile/glacial acetic acid (108:72:20:1 v/v/v/v), eluting at a flow rate of 1 ml/min. The flow rate of the scintillation fluid was 3 ml/min, and the efficiency of the radiodetector cell was 77%.

Standard riluzole samples were prepared in phosphate buffer and mixed with methanol/acetonitrile as for the incubation samples. UV

![Fig. 1. Enzyme kinetics of riluzole N-hydroxylation and glucuronidation by human hepatic microsomes and by yeast microsomes expressing human CYP1A isoenzymes. Top left, saturation curve for riluzole hydroxylation by human hepatic microsomes. Top right, saturation curve for riluzole hydroxylation by yeast microsomes expressing human CYP1A2. Bottom left, saturation curve for riluzole glucuronidation by human hepatic microsomes. Bottom right, saturation curve for riluzole glucuronidation by yeast microsomes expressing human CYP1A1. Insets, Eadie-Hofstee transformations of the same data: saturation functions were obtained by fitting untransformed data to a simple Michaelis-Menten function using iterative nonlinear regression analysis.](image-url)
detection of riluzole and its metabolites was linear over the concentration range of 1 to 100 μM. The radiodetector, which was calibrated by comparing [14C]riluzole peak areas with radioactivity counts in a Beckman LS 6000SC liquid scintillation counter, yielded linear detection over a concentration range of 1.35 to 500 μM.

Data Analysis

The kinetic parameters of riluzole metabolism [Vmax, apparent Ki, and IC50 (defined as the inhibitor concentration reducing riluzole hydroxylation by 50%)] were calculated by iterative nonlinear regression analysis using GraFit Version 3.0 software. Intrinsic metabolic clearance (Clint) was calculated as Vmax/Km. Results are expressed as mean ± S.E.M.

Results

Riluzole Monooxidation

Enzyme kinetics. [14C]Riluzole was metabolized in an NADPH-dependent (monooxygenase-catalyzed) manner on incubation with human hepatic microsomes, resulting in the formation of the N-hydroxylated derivative. The mean rate of riluzole N-hydroxylation by hepatic microsomes from 6 individuals was 138 ± 53 pmol/min/mg. The rate increased linearly with microsomal protein concentration up to 2.5 mg/ml. The reaction followed normal single-enzyme Michaelis-Menten kinetics (fig. 1); apparent kinetic parameters Vmax and Km were calculated with the CYP2C19 substrate omeprazole (31% inhibition at 5 μM) but also with the negative control, quinine (table 2). Tolbutamide (a CYP2C9 substrate), sulfaphenazol (a CYP2C9 inhibitor), mephenytoin (a CYP2C19 substrate), ketoconazole and troleandomycin (CYP3A4 inhibitors) had no appreciable effect on riluzole N-hydroxylation.

TABLE 2

<table>
<thead>
<tr>
<th>Cytochrome P450 isoenzyme</th>
<th>Substrate/inhibitor</th>
<th>Concentration</th>
<th>Inhibition Riluzole</th>
<th>N-Hydroxylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2/2</td>
<td>α-Naphthoflavone</td>
<td>1</td>
<td>80</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>400</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Caffeine</td>
<td>1000</td>
<td>37</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Acetanilide</td>
<td>1000</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Ketoconazole</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Quinidine</td>
<td>300</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Quinine (control)</td>
<td>5</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>CYP2E6</td>
<td>Quinidine</td>
<td>100</td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Chloroxazone</td>
<td>100</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>p-Nitrophenol</td>
<td>100</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Aniline</td>
<td>50</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>CYP2C8/9</td>
<td>Tolbutamide</td>
<td>50</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Omeprazole</td>
<td>100</td>
<td>31</td>
<td>24</td>
</tr>
</tbody>
</table>

Some inhibition of riluzole N-hydroxylation was observed with the CYP2C19 substrate omeprazole (31% inhibition at 100 μM) and with the CYP2D6 substrate quinidine (20% inhibition at 5 μM) but also with the negative control, quinine (table 2). Tolbutamide (a CYP2C8/9 substrate), sulfaphenazol (a CYP2C9 inhibitor), mephenytoin (a CYP2C19 substrate), ketoconazole and troleandomycin (CYP3A4 inhibitors) had no appreciable effect on riluzole N-hydroxylation.

Enzyme source Cosubstrate Vmax Km Clint

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Cosubstrate</th>
<th>Vmax (μM)</th>
<th>Km (μM)</th>
<th>Clint (ml/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic microsomes</td>
<td>NAPDH</td>
<td>513 ± 36a</td>
<td>29.7 ± 3.5</td>
<td>17.28a</td>
</tr>
<tr>
<td>CYP1A2-yeast microsomes</td>
<td>NAPDH</td>
<td>2.24 ± 0.12b</td>
<td>25.7 ± 2.2</td>
<td>0.092b</td>
</tr>
<tr>
<td>CYP1A1-yeast microsomes</td>
<td>NAPDH</td>
<td>7.61 ± 0.34b</td>
<td>6.24 ± 0.55</td>
<td>1.22b</td>
</tr>
<tr>
<td>Hepatic microsomes</td>
<td>UDPGA</td>
<td>62.0 ± 10.5a</td>
<td>118 ± 27</td>
<td>0.53a</td>
</tr>
</tbody>
</table>

Clint = intrinsic metabolic clearance.

Values are mean ± S.E.M.
imipramine and quinine (all at 1 mM) also caused >50% inhibition of riluzole N-hydroxylation. Intermediate inhibition (25–50%) was observed with theophylline, cimetidine, caffeine, ranitidine and paracetamol, whereas minimal inhibition (<10%) was seen with thiamine, captopril, pefloxacin, aspirin, amoxicillin, metronidazole, piracetam and baclofen at concentrations of 1 mM. Sparfloxacin (≥400 μM) had no effect on parent riluzole biotransformation but did reduce levels of N-hydroxyriluzole, suggesting that it may react directly with this metabolite rather than with the parent compound.

**Effect of riluzole on P450 enzyme activities.** Riluzole at 1 and 10 μM had a weak inhibitory effect on human hepatic microsomal CYP1A2-mediated phenacetin O-deethylation, CYP2A6-mediated coumarin-7-hydroxylation, CYP2D6-mediated bufuralol-1-hydroxylation and CYP2E1-mediated chlorzoxazone-6-hydroxylation (table 4). Only the effects on phenacetin O-deethylation and chlorzoxazone-6-hydroxylation were concentration dependent. Because riluzole is metabolized by the CYP1A2 isoenzyme, we studied the inhibition kinetics of CYP1A2-catalyzed methoxyresorufin-O-demethylation. Riluzole competitively inhibited methoxyresorufin-O-demethylation with an inhibition constant ($K_i$) of 12.1 ± 1.5 μM in human liver microsomes and 16.7 ± 1.4 μM in microsomes from CYP1A2-expressing yeast (fig. 3). No appreciable or consistent inhibition of CYP2C9-catalyzed tolbutamide 4-hydroxylation, CYP2C19-catalyzed S-mephenytoin 4-hydroxylation or CYP3A4-catalyzed nifedipine dehydrogenation was seen with riluzole.

**Riluzole Glucuronidation**

**Enzyme kinetics.** Riluzole was metabolised in an UDPGA-dependent (i.e., UGT catalyzed) manner on incubation with detergent-activated human hepatic microsomes, resulting in the formation of a single unidentified metabolite. The reaction followed normal single-enzyme Michaelis-Menten kinetics (fig. 1); apparent Michaelis-Menten kinetic parameters $V_{max}$ and $K_m$ are shown in table 1.

**Inhibition of riluzole glucuronidation**

Riluzole glucuronidation was inhibited in a concentration-dependent manner by preincubation with propofol ($IC_{50}$ = 18.7 μM), indicating the involvement of the UGT HP4 (UGT1.8/9)
TABLE 4

Effects of riluzole (1 and 10 \( \mu \)M) on human hepatic cytochrome P450-mediated oxidative drug metabolism

<table>
<thead>
<tr>
<th>Drug oxidation</th>
<th>P450 isozyme</th>
<th>1 ( \mu )M riluzole</th>
<th>10 ( \mu )M riluzole</th>
<th>1 ( \mu )M riluzole</th>
<th>10 ( \mu )M riluzole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenacetin-O-deethylation</td>
<td>CYP1A2</td>
<td>+5</td>
<td>-19</td>
<td>0</td>
<td>-12</td>
</tr>
<tr>
<td>Coumarin-7-hydroxylation</td>
<td>CYP2A6</td>
<td>-13</td>
<td>-17</td>
<td>-2</td>
<td>-2</td>
</tr>
<tr>
<td>Tolbutamide-4-hydroxylation</td>
<td>CYP2C9</td>
<td>+6</td>
<td>+3</td>
<td>+10</td>
<td>+4</td>
</tr>
<tr>
<td>S-Mephenytoin-4-hydroxylation</td>
<td>CYP2C19</td>
<td>+1</td>
<td>-2</td>
<td>-1</td>
<td>+3</td>
</tr>
<tr>
<td>Bufuralol-1-hydroxylation</td>
<td>CYP2D6</td>
<td>-9</td>
<td>-14</td>
<td>-11</td>
<td>-14</td>
</tr>
<tr>
<td>Chlorzoxazone-6-hydroxylation</td>
<td>CYP2E1</td>
<td>-5</td>
<td>-28</td>
<td>-7</td>
<td>-20</td>
</tr>
<tr>
<td>Nifedipine dehydrogenation</td>
<td>CYP3A4</td>
<td>-15</td>
<td>+4</td>
<td>-4</td>
<td>+8</td>
</tr>
</tbody>
</table>

* Substrate concentrations: nifedipine, 25 and 50 \( \mu \)M; chlorzoxazone, 40 and 80 \( \mu \)M; bufuralol, 15 and 30 \( \mu \)M; S-mephenytoin, 100 and 200 \( \mu \)M; tolbutamide, 125 and 250 \( \mu \)M; coumarin, 0.5 and 1 \( \mu \)M; phenacetin, 40 and 100 \( \mu \)M.

\( ^{+} \): Enhancement of drug metabolism; \( ^{-} \): inhibition of drug metabolism.

Results are expressed as the mean values for two human liver samples.

Discussion

Two types of human hepatic enzyme are involved in the biotransformation of riluzole by the human liver in vitro: monoxygenases and UDP-glucuronosyltransferases. Quantitatively, monoxygenation is possibly the more important reaction because intrinsic clearance \( \text{via} \) this route was 30-fold higher than that \( \text{via} \) direct glucuronidation.

Riluzole N-hydroxylation was the only monooxygenase-mediated reaction observed with human hepatic microsomes. CYP1A2 appears to be the main isozyme involved in this reaction, a conclusion that is based on the potent inhibition observed with the specific CYP1A2 inhibitor \( \alpha \)-naphthoflavone (IC\(_{50} = 0.42 \) \( \mu \)M), the specific biotransformation noted with genetically expressed CYP1A2, and the competitive inhibitory effect of riluzole on CYP1A2-catalyzed methoxyresorufin O-demethylation. The relatively weak inhibitory effect of the CYP1A2 substrates caffeine and acetaminidile on riluzole N-hydroxylation may be attributed to the low affinity of these compounds for CYP1A2 (\( K_{m} = 0.5 \) to 1.5 mM) (Grant et al., 1987) compared with that of riluzole (\( K_{m} = 23 \) \( \mu \)M). In keeping with the present finding, the CYP1A2 isozyme has previously been shown to catalyze specifically the N-hydroxylation of many heterocyclic (aryl) amines in humans (Boobis et al., 1994; Gonzalez and Idle, 1994). Because CYP1A2 seems to be the only CYP1A isozyme expressed in human liver (Gonzalez, 1992), it can be concluded that this is the major isozyme involved in the hepatic metabolism of riluzole. CYP1A1 isozymes are readily induced \( \text{in vivo} \) by tobacco smoke (Guengerich and Shimada, 1991). Interestingly, increased riluzole clearance in smokers has been demonstrated in a recent population pharmacokinetic study of riluzole.\(^{+}\)

Possible extrahepatic metabolism of riluzole is suggested by the finding that microsomes of genetically engineered yeast cells expressing human CYP1A1 catalyzed the formation of several hydroxylated derivatives that have previously been identified in the urine of patients treated with riluzole.\(^{2}\) The CYP1A1 isozyme is expressed largely in extrahepatic tissue, such as the lung (Gonzalez, 1992).

Although the disparate effects of the various CYP2E1 substrates on riluzole N-hydroxylation appear somewhat contradictory (some inhibition occurring with chlorzoxazone, but not with aniline, isoniazid or p-nitrophenol), it should be noted that not all these substrates are highly specific for CYP2E1. Thus, although chlorzoxazone and p-nitrophenol share similarly high affinities (\( K_{m} \approx 30 \) \( \mu \)M) for CYP2E1 (Peter et al., 1991; Tassaneeyakul et al., 1993a), chlorzoxazone is also metabolized by CYP1A2 (Ono et al., 1996). The lack of inhibitory effect of p-nitrophenol at concentrations as high as 1 mM and the absence of metabolism by CYP2E1 expressed in B-lymphoblastoid cells suggest that this isoenzyme does not play an appreciable role in riluzole oxidation.

The use of in vitro systems such as human hepatic microsomes in drug-interaction studies is recommended for predicting the consequences of concurrent drug therapy (Peck et al., 1993). As a substrate for specific P450 isoforms, riluzole has the potential to act as a competitive enzyme inhibitor and thereby alter the metabolism and pharmacokinetics of coadministered drugs that are also subject to phase I metabolism. Effectively, riluzole is a competitive inhibitor of CYP1A2-catalyzed methoxyresorufin O-demethylation, with a \( K_i \) value close to its \( K_m \) value. At in vitro concentrations of 1 and 10 \( \mu M \), similar to or higher than those achieved therapeutically, \(^2\) riluzole had a weak inhibitory effect on human hepatic microsomal CYP1A2-, CYP2A6-, CYP2D6- and CYP2E1-mediated oxidative drug metabolism. Apart from inhibition of methoxyresorufin O-demethylation, the most pronounced, concentration-dependent inhibition (28%) was that of CYP2E1-catalyzed chlorozoxazone-6-hydroxylation. However, as mentioned above, this inhibition probably reflects on CYP1A2 as well as CYP2E1. Inhibition by riluzole of microsomal CYP1A2-catalyzed phenacetin O-deethylation is not unexpected given the evidence for the involvement of this isoenzyme in riluzole metabolism. However, the weak inhibitory effect of riluzole suggests that it is unlikely to alter to any appreciable extent the hepatic clearance of drugs that are oxidized by the CYP system.

Not surprisingly, known drug substrates of CYP1A2, including enoxacin (Edwards et al., 1988), cimetidine (Knodell et al., 1991), paracetamol (Raucy et al., 1989), imipramine (Lemoine et al., 1993) and the methylxanthines caffeine and theophylline (Fuhr et al., 1992; Tassaneeyakul et al., 1993b), had an inhibitory effect (IC\(_{50}\) values \( \geq 400 \) \( \mu M \)) on riluzole N-hydroxylation. In contrast to enoxacin, and in keeping with their lack of effect on theophylline metabolism in vivo or in vitro (Edwards et al., 1988), the quinolones pefloxacin and sparfloxacin had no direct effect on riluzole hydroxylation. The inhibitory effect of cimetidine, a well known CYP inhibitor in vivo (Smith and Kendall, 1988), was comparatively weak (IC\(_{50}\) = 937 \( \mu M \)). However, it has previously been noted that cimetidine inhibition can be underestimated in vitro, possibly because its interaction with CYP proceeds rather slowly (Chang et al., 1992).

Although the enzymes responsible for clomipramine and amitriptyline metabolism have not been identified, both these tricyclic antidepressants are susceptible to interaction with fluvoxamine, a potent CYP1A2 inhibitor (Berchty et al., 1991; Brosen et al., 1993). Moreover, both imipramine and amitriptyline have been shown to be mechanism-based inhibitors of CYP (Murray and Field, 1992), so an effect of these drugs on riluzole metabolism is not unexpected.

For all the tested drugs, the IC\(_{50}\) value was \( \geq 14 \) times greater than the riluzole concentration (15 \( \mu M \)) in the incubate. Therefore, inhibition of riluzole metabolism appears a priori unlikely, but results from in vivo drug-interaction studies are required before it can be concluded that these agents effectively inhibit riluzole metabolism or alter its pharmacokinetics in humans.

Knowledge of UGT isoenzymes and their substrate specificity is much more limited than is the case with the CYP system. Nevertheless, using genetically expressed enzymes, Ebner and Burchell (1993) established that within the UGT1 gene family, propofol is a specific substrate for UGT HP4 (UGT1.8/9), whereas 1-naphthol is more specific for UGT HP1 and bilirubin is specific for UGT HP2 and UGT HP3. Therefore, in view of the pronounced inhibitory effect of propofol and the minimal effect of 1-naphthol and bilirubin, riluzole conjugation is most likely to be mediated by the UGT HP4 isoenzyme. Although several other compounds, many of them substrates for UGT2 isoenzymes (androsterone, etilhydroxylic, inhibited riluzole glucuronidation to varying extents, lack of substrate specificity and the low biotransformation rate of riluzole make it difficult to evaluate the significance of these findings.

In conclusion, riluzole is predominantly metabolized by CYP1A2 in human hepatic microsomes, whereas extrahepatic CYP1A1 is also responsible for the formation of several human metabolites that are also observed in vivo. The fact that riluzole is a specific substrate for the CYP1A2 isoenzyme, has a single oxidative metabolic pathway in the liver and is a nontoxic drug with low metabolic clearance in humans could make it an interesting candidate as an in vitro and in vivo probe. This is further demonstrated by the effect of tobacco use on riluzole clearance in patients with ALS. Direct glucuronidation is a relatively minor metabolic route and is catalyzed by UGT HP4. On the basis of in vitro findings, at therapeutic doses riluzole is unlikely to alter the pharmacokinetics of coadministered drugs that undergo phase I metabolism. Conversely, significant modification of the pharmacokinetics of riluzole by these drugs would not be anticipated in clinical practice, although this has yet to be confirmed.

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