ELETRIPATAN METABOLISM BY HUMAN HEPATIC CYP450 ENZYMES AND TRANSPORT BY HUMAN P-GLYCOPEPTIDE

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(Received December 23, 2002; accepted April 9, 2003)

ABSTRACT:

“Reaction phenotyping” studies were performed with eletriptan (ETT) to determine its propensity to interact with coadministered medications. Its ability to serve as a substrate for human P-glycoprotein (P-gp) was also investigated since a central mechanism of action has been proposed for this “triptan” class of drug. In studies with a characterized bank of human liver microsome preparations, a good correlation (r² = 0.932) was obtained between formation of N-desmethyl eletriptan (DETT) and CYP3A4-catalyzed testosterone 6β-hydroxylation. DETT was selected to be monitored in our studies since it represents a significant ETT metabolite in humans, circulating at concentrations 10 to 20% of those observed for parent drug. ETT was metabolized to DETT in recombinant CYP2D6 (rCYP2D6) and rCYP3A4, and to a lesser extent by rCYP2C9 and rCYP2C19. The metabolism of ETT to DETT in human liver microsomes was markedly inhibited by tropoendomycin, erythromycin, miconazole, and an inhibitory antibody to CYP3A4, but not by inhibitors of other major P450 enzymes. ETT had little inhibitory effect on any of the P450 enzymes investigated. ETT was determined to be a good substrate for human P-gp in vitro. In bidirectional transport studies across LLC-MDR1 and LLC-Mdr1a cell monolayers, ETT had a BA/AB transport ratio in the range 9 to 11. This finding had significance in vivo since brain exposure to ETT was reduced 40-fold in Mdr1a+/− mice relative to Mdr1a−/− mice. ETT metabolism to DETT is therefore catalyzed primarily by CYP3A4, and plasma concentrations are expected to be increased when coadministered with inhibitors of CYP3A4 and P-gp activity.

Eletriptan [ETT1; (R)-3-(1-methyl-2-pyrrolidinylmethyl)-5-[2-(phenylsulfonyl)ethyl]-1H-indole] (Fig. 1) is a selective agonist at 5-hydroxytryptamine (serotonin)1H/1D receptors and, like the other indole-3-(1-methyl-2-pyrrolidinylmethyl)-5-[2-(phenylsulfonyl)ethyl]-1H-indole] (Fig. 1) is a selective agonist at 5-hydroxytryptamine (serotonin)1H/1D receptors and, like the other drugs in this “triptan” class, has been shown to be clinically effective for the treatment of migraine (Jackson, 1996). The pharmacokinetics and metabolism of ETT have been investigated in the rat, dog, and human. In all three species, ETT was rapidly absorbed and extensively cleared by metabolism (Milton et al., 1997; Morgan et al., 2000). The pathways of ETT metabolism are similar in the rat, dog, and human, and principal routes include pyrrolidine N-demethylation to N-desmethyl eletriptan (DETT), together with N-oxidation, oxidation of the pyrrolidine ring, and formation of tetracyclic quaternary ammonium metabolites (Morgan et al., 2000).

Members of the cytochrome P450 (P450) superfamily are known to play a major role in the oxidative metabolism of both xenobiotics and certain endogenous compounds. Described herein are the first detailed studies to elucidate the P450 forms responsible for ETT metabolism to DETT in human liver. The metabolism of ETT to DETT was chosen to be monitored in our studies since DETT represents a significant metabolite observed to circulate in humans at levels 10 to 20% of those observed for ETT [Product Information for Eletriptan, Pfizer, Sandwich, UK (2001)]. Reaction phenotyping studies (Rodrigues, 1999) were performed by correlation analysis, the use of recombinant human P450 forms, chemical inhibition, and inhibitory antibodies. In addition, to evaluate the potential for drug-drug interactions, the effect of ETT on some selected P450 form enzyme activities was also investigated.

It was of interest also to determine whether ETT could serve as a substrate for P-glycoprotein (P-gp), an efflux transporter expressed on the apical side of gut epithelia and brain endothelia, which can limit oral absorption and brain penetration, respectively. The role of P-gp and P450 in the uptake and metabolism of ETT was investigated because these systems can work in concert to limit systemic exposure following oral dosing (Cummins et al., 2002), and they are also subject to inhibition and/or induction by a variety of xenobiotics (Wandel et al., 1999; Dresser et al., 2003). In addition, the role of P-gp in regulating the uptake of ETT into the brain was of interest because of the intense speculation about a central mechanism of action for the triptan class of antimigraine drugs (Goadsby and Hargreaves, 2000). In this regard, ETT was considered the ideal tool to investigate this...
phenomenon, since it was the first lipophilic agent in this drug class that would be expected to have good intrinsic brain penetration. Our studies describe primarily the role of P-gp and P450 in the disposition of ETT from the perspective of its potential for drug-drug interactions. The role of P-gp in potentially modulating the dose required for antimigraine efficacy is also discussed in the context of a comparison to the other triptan drugs, naratriptan (NARA), rizatriptan (RIZA), sumatriptan (SUMA), and zolmitriptan (ZOLM).

**Materials and Methods**

**Materials.** ETT, NARA, RIZA, SUMA, ZOLM (Fig. 1), DETT, and L-844151 [internal standard for liquid chromatography/tandem mass spectrometry (LC/MS-MS)] were synthesized by Merck Sharpe & Dohme (UK) with identity and purity confirmed by NMR, MS, HPLC, and elemental analysis. NADPH, Tris, diethyldithiocarbamate, erythromycin, miconazole, sulfaphenazole, troleandomycin, 7-ethoxyresorufin, resorufin, tolbutamide, debrisoquine, chlorzoxazone, testosterone, and 6- and 7-hydroxytestosterone were obtained from Sigma-Aldrich Company Ltd. (Poole, Dorset, UK); 862 EVANS ET AL.

**Human Liver Microsomes.** Washed microsomal fractions were prepared in 0.154 M KCl containing 50 mM Tris-HCl, pH 7.4, using standard methods. Two separate batches, designated pools I and II, of pooled human liver microsomes were prepared. Each batch was prepared by pooling liver samples from five subjects. Pool I comprised liver samples from males aged 2.5 and 58 years and females aged 14, 59, and 74 years, whereas pool II comprised liver samples from male subjects aged 44 and 50 years and female subjects aged 11, 31, and 55 years.

**Metabolism of ETT by Human Liver Microsomes.** The NADPH-dependent metabolism of ETT was studied in incubations containing 0.05 to 200 μM ETT (added in 0.25 ml of 0.1 M phosphate buffer, pH 7.4), 10 mM MgCl2, 2 mM EDTA, 85 mM phosphate buffer, pH 7.4, and 0.05 to 0.3 mg of microsomal protein in a final volume of 0.5 ml. After a 5-min preincubation at 37°C in a shaking water bath, the reaction was initiated by the addition of 1 mM NADPH. Incubations were performed in duplicate for 5 to 20 min at 37°C and were terminated by the addition of 0.5 ml of ice-cold acetonitrile containing the L-844151 internal standard for LC/MS-MS analysis (see below). Blank incubations contained all components except NADPH.

**ETT Metabolism to DETT was quantified by LC/MS-MS analysis.** An HPLC PAL autosampler (Presearch Ltd., Hitchin, Hertfordshire, UK) attached to an HP1100 series high-performance liquid chromatograph (Hewlett Packard Ltd., Bracknell, Berkshire, UK) were used to introduce samples into a Micromass Quattro LC triple quadrupole mass spectrometer (Micromass UK Ltd., Altrincham, Cheshire, UK) using an Electrospray Z-spray source. All incubations were terminated by the addition of an equal volume of ice-cold acetonitrile containing 200 μM L-844151 internal standard. After vortex mixing, the tubes were centrifuged at 10,000g for 10 min at 4°C. Chromatography of 20-μl aliquots of the supernatant was performed with a 150 x 3.0 mm Kromasil C18 5-μm column (Thermo Hypersil-Keystone, Runcorn, Cheshire, UK) and isocratic elution with a mobile phase of 40% (v/v) acetonitrile, 6% (v/v) 250 mM ammonium formate, pH 3.0 and 54% (v/v) ultrapure water, at a flow rate of 0.4 ml/min and a column temperature of 35°C. The eluate was split volumetrically, with 20% passing directly into the triple quadrupole mass spectrometer operating in positive electrospray mode, with probe and source block temperatures of 400 and 100°C, respectively, using nitrogen for desolvation and nebulization, and a capillary voltage of 2.8 kV to initiate ionization. The compounds were selectively detected by multiple reaction monitoring using argon, collision energy 21 V; ETT m/z 383→84, DETT m/z 369→70, and L-844151 internal standard m/z 354→113.

**ETT Metabolism Correlation Analysis.** Incubation mixtures contained 60 μM ETT (added in 0.25 ml of 0.1 M phosphate buffer, pH 7.4), 10 mM MgCl2, 2 mM EDTA, 85 mM phosphate buffer, pH 7.4, and 0.1 mg of microsomal protein in a final volume of 0.5 ml. After 5 min of preincubation at 37°C in a shaking water bath, the reaction was initiated by the addition of 1 mM (final concentration) NADPH. Incubations were conducted in triplicate with a single blank (no NADPH) for each of the 16 preparations of characterized human liver microsomes and were terminated after 5 min by the addition of 0.5 ml of

![Fig. 1. Structures of 5-HT1B/1D agonists.](https://example.com/fig1.png)
ice-cold acetonitrile containing the L-844151 internal standard. Incubations were processed for LC/MS-MS analysis as described above.

**ETT Metabolism by Recombinant P450 Forms.** Incubation mixtures contained 60 μM ETT (added in 0.125 ml of 0.1 M phosphate buffer, pH 7.4) 10 mM MgCl₂, 2 mM EDTA, 1 mM NADPH, and 90 mM phosphate buffer, pH 7.4, in a final volume of 0.25 ml. After a 5-min preincubation at 37°C in a shaking water bath, the reaction was initiated by adding 0.1 mg of B-

lymphoblastoid cell microsomal protein with gentle mixing. The incubations were conducted in duplicate with duplicate blank (no microsomes) for each P450 form preparation. Reactions were terminated after either 5 or 30 min with 0.25 ml of ice-cold acetonitrile containing the L-844151 internal standard.

Incubations were processed for LC/MS-MS analysis as described above.

**ETT Metabolism Inhibition Studies.** Apart from the studies with the CYP1A2 mechanism-based inhibitor furafylline (Newton et al., 1995), where dimethyl sulfoxide was used, methanol was used as the solvent for these investigations to minimize solvent effects on individual P450 forms.

For the mechanism-based inhibitors requiring preincubation with NADPH, incubation mixtures contained 10 mM MgCl₂, 2 mM EDTA, 1 mM NADPH, 84 mM phosphate buffer, pH 7.4, 0.1 mg of microsomal protein, and either 5 to 50 μM furafylline, 5 to 100 μM diethyldithiocarbamate, 2 to 50 μM troleandomycin, or 2 to 50 μM erythromycin in a volume of 0.25 ml. The inhibitors were added in either methanol (5 μL/tube) or dimethyl sulfoxide (furafylline only, 5 μL/tube), and the concentrations refer to the final concentrations in a 0.5-ml incubation. Following a 30-min preincubation at 37°C in a shaking water bath, 60 μM ETT and 1 mM NADPH (final concentration 2 mM ETT) were added in 0.25 ml of 0.1 M phosphate buffer, pH 7.4, at 37°C. Incubations were performed in duplicate for 5 min at 37°C and were terminated by the addition of 0.5 ml of ice-cold acetonitrile containing the L-844151 internal standard. Incubations were processed for LC/MS-MS analysis as described above.

For compounds not requiring extensive preincubation with NADPH, incubation mixtures contained 10 mM MgCl₂, 2 mM EDTA, 1 mM NADPH, 84 mM phosphate buffer, pH 7.4, 0.1 mg of microsomal protein, and either 2 to 50 μM sulfaphenazole, 2 to 20 μM quinidine, 50 to 500 μM S-mephenytoin, or 2 to 50 μM miconazole, in a volume of 0.25 ml. All the inhibitors were added in methanol (5 μL/tube), and the concentrations refer to the final concentrations in a 0.5-ml incubation. After a 10-min preincubation at 37°C in a shaking water bath, the reaction was initiated by the addition of 60 μM ETT (added in 0.25 ml of 0.1 M phosphate buffer, pH 7.4, at 37°C). Incubations were performed in duplicate for 5 min at 37°C and were terminated by the addition of 0.5 ml of ice-cold acetonitrile containing the L-844151 internal standard.

Incubations were performed for LC/MS-MS analysis as described above.

To study the effect of inhibitory antibodies to CYP2C8/9, CYP2D6, and Mdr1a (Fischer et al., 1997) on the brain entry of the triptan drugs was investigated in vivo using male C57BL/6 mice. ETT was formulated in 25% polyethylene glycol 300/water at 0.5 mg free base equivalents/ml, and NARA, RIZA, ZOLM, and SUMA were formulated as aqueous solutions at the same concentration. All drugs were dosed at 1 mg free base equivalents/kg as a bolus injection into the tail vein (dose volume 2 ml/kg) to 24 male C57-1 mice (12 Mdr1a+/−, 12 Mdr1a−/−), approximate weight 35 g. Blood (by cardiac puncture into heparinized containers) and brain samples were taken at either 0.5, 2, 4, or 6 h after dosing (n = 3 per time point per genotype). Samples were stored frozen (-80°C) until analysis.

To blood (0.1 ml) was added internal standard (10 μl of a 10 ng/μl solution), 1 M sodium hydroxide (0.05 ml), water (1 ml), and ethyl acetate (4 ml). Samples were vortex mixed and centrifuged. Supernatant was removed and evaporated to dryness (70°C, under nitrogen), and the residue was dissolved in mobile phase (0.1 ml) and transferred to a HPLC vial. Calibration standards covering appropriate ranges were prepared by spiking solutions of analyte at appropriate concentrations into control blood (0.1 ml).

To weighed samples of brain cortex were added internal standard (10 ng/μl solution) and water (2 ml). After homogenization with an ultrasonic probe, 1 M sodium hydroxide (0.2 ml) and ethyl acetate (4 ml) were added. Samples were vortex mixed and centrifuged. Supernatant was removed and evaporated to dryness (70°C, under nitrogen), and the residue was dissolved in mobile phase (0.15 ml) and transferred to a HPLC vial. Calibration standards covering appropriate ranges were prepared by spiking solutions of analyte at appropriate concentrations into control brain cortex.

Typically 25-μl injections were made onto a KR100-SC8 HPLC column (150 × 3.2 mm i.d., Hichrom Ltd., Reading, UK) with a mobile phase consisting of acetonitrile (A) and 25 mM ammonium formate, adjusted to pH 3 with formic acid, at a flow rate of 0.4 ml/min, with the following time program: 0 min, 20% A; 2 min, 20% A; 6 min, 50% A; 7 min, 20% A; 10 min, 20% A. Detection was for appropriate fragmentation transitions as determined by infusion of 1 ng/μl solutions at 10 ml/h into the mass spectrometer (Micromass Quattro).

Since three animals per time point were used to construct blood and brain concentration versus time profiles, Baillier’s method for determining area under
the curve (AUC) confidence intervals during sparse sampling was applied (Bailier, 1988; Nedelman et al., 1995). Standard error of the mean (S.E.M.) for the brain/blood ratio was determined using the following equation: $S.E.M. = (Brain\ AUC\ S.E.M. /Blood\ AUC^2) + (Brain\ AUC^2 / Blood\ AUC^3)$. An unpaired two-tailed test was performed to determine the significance of the difference between brain/blood AUC$^{0.5}$ and Blood AUC$^{4}$. An unpaired two-tailed test was performed to determine the significance of the difference between brain/blood AUC$^{2}$ S.E.M. 2 and Blood AUC$^{2}$/Blood AUC$^{2}$ in Mdr1a$^{-/-}$ and Mdr1a$^{+/+}$ mice.

**Human MDR1 and Mouse Mdr1a P-Glycoprotein Bidirectional Transport Assay.** The extent of P-gp-mediated transport of ETT, NARA, RIZA, ZOLM, and SUMA was also evaluated in the following: 1. LLC-PK1 wild-type, LLC-MDR1 (human), and LLC-Mdr1a (mouse) cell lines (kindly provided by Dr. A. H. Schinkel, The Netherlands Cancer Institute, Amsterdam, The Netherlands). Cells were cultured in 199 medium as described. For transport experiments, cells were expressed in 24-well Transwell plates (Falcon) at a density of $1.5 \times 10^5$ cells/well with 0.3 ml of medium in the upper compartment and 35 ml of medium in the lower nonsubdivided compartment. After culturing for 5 days, the transport wells were transferred to a 24-well plate. The incubation and sampling procedure during the transport experiment was performed on a Tecan Genesis Workstation 150 Robot (Tecan, Zürich, Switzerland). Cells were washed three times with Hanks balanced salt solution containing Hepes (10 mM). Subsequently, analyte in Hanks balanced salt solution containing Hepes (500 μl) was added either to the apical or basolateral compartment. Analyte concentrations used were: 10 μM ETT, NARA, RIZA, ZOLM, and SUMA, 10 μM dexamethasone, 5 μM rianivavir, and 0.5 μM verapamil. After incubating for 4 h at 37°C, samples (100 μl) were taken from both the apical and basolateral compartment and transferred to a 96-well microtiter plate. Samples were analyzed by positive ion single mono ion monitoring atmospheric pressure chemical ionization on a SCIEX API 2000 triple quadrupole mass spectrometer (PerkinElmerSciex, Concord, ON, Canada). The HPLC system used was a PerkinElmer Series 200 system with 2 Micro pumps (PerkinElmer Instruments, Shelton, CT) and a Leap HTS PAL autosampler (LEAP Technologies, Carrboro, NC). Samples were chromatographed using an Aquasil C18, 2 × 20 mm, 5-μm HPLC column (Thermo Hypersil, Keystone Scientific Operations, Bellefonte, PA). The mobile phase was used 0.1% acetic acid in water (A) or in acetonitrile (B) at 2.0 ml/min with a 4:1 split. The HPLC run time was 2 min/sample. Standard curves were prepared by a 2-fold serial dilution in water/methanol (60:40, v/v) of a standard prepared at 2 times the dosing solution concentration for an 11-point standard curve. Percentage of transport was calculated by dividing the concentration of the compound appearing in the lower compartment (resulting from introduction of compound in the apical compartment) by the concentration of compound in the apical compartment (resulting from introduction of compound in the basolateral compartment). The BA/AB ratio was calculated by dividing the concentration of compound measured in the apical compartment (resulting from introduction of compound in the basolateral compartment) by the concentration of compound measured in the apical compartment (resulting from introduction of compound in the apical compartment). The effects of the mechanism-based inhibitors 5 to 50 μM furafylline (●) and 5 to 100 μM diethylthiocarbamate (○) (A), together with 2 to 50 μM sulfaphenazole (■), 2 to 20 μM quinidine (▲), and 50 to 500 μM S-mephenytoin (△) (B) were evaluated on the metabolism of 60 μM ETT to DETT. Incubations were performed with 0.1 mg protein of the pool I human liver microsomal preparation for 5 min. Each point represents the mean of a duplicate test incubation.

**Lipophilicity Measurements.** Octanol-pH 7.4 buffer partition coefficients (logD$^0_{o/w}$) were determined by the shake flask method with HPLC-UV analysis of the aqueous and organic phases after equilibration (Hansch and Leo, 1979). Results were derived from the mean of two partition coefficient determinations performed at overall concentrations of 0.5 and 0.25 mg/ml in 1:1 pH 7.4 buffer/octanol mixtures.

**Results**

**Metabolism of ETT by Human Liver Microsomes.** Studies were performed with two pools, namely pool I and pool II, of human liver microsomes. The total P450 content of the pool I and II microsomal preparations was determined to be 0.72 and 0.51 nmol/mg protein, respectively. The linearity of 50 μM ETT metabolism with respect to incubation time and protein concentration was investigated initially. ETT metabolism to DETT was found to be linear with respect to incubation time (up to at least 6 min) and protein concentration (up to at least 0.2 mg of protein/incubation).

**Kinetics of ETT Metabolism in Human Liver Microsomes.** The kinetics of the NADPH-dependent metabolism of 0.05 to 200 μM ETT to DETT was examined with both the pool I and pool II human
liver microsomal preparations. Pooled, rather than individual, liver microsomal preparations were used for these studies since the aim was to identify average $K_m$ (i.e., the concentration of substrate giving half-maximal velocity) values to select a suitable substrate concentration for the reaction phenotyping studies. With both liver microsome pools, DETT formation was detected at all ETT substrate concentrations examined.

The kinetics of ETT metabolism to DETT was examined with Michaelis-Menten and Eadie-Hofstee plots. Kinetic analysis of data for both liver microsome pools revealed mean $K_m$ and $V_{max}$ (i.e., the maximal velocity) values of 27.1 $\mu$M (pool I and pool II individual values were 24.3 and 29.9 $\mu$M, respectively) and 78 pmol/min/mg protein (pool I and pool II individual values were 105.4 and 50.5 pmol/min/mg protein, respectively). The mean intrinsic clearance ($CL_{int}$; i.e., $V_{max}/K_m$) value was 3.02 $l/min/mg$ protein (pool I and pool II individual values were 4.34 and 1.69 $l/min/mg$ protein).

An ETT substrate concentration of 60 $\mu$M was selected for the reaction phenotyping studies described below. This substrate concentration was selected as being approximately 2 times the $K_m$ value for DETT formation.

**ETT Metabolism Correlation Analysis.** The metabolism of 60 $\mu$M ETT to DETT was examined with a characterized panel of 16 human liver microsomal preparations. ETT was metabolized to DETT by all 16 human liver microsomal preparations examined. Microsomal ETT metabolism varied from 58 to 635 pmol/min/mg protein, with most of the individual human liver microsomal preparations being more active than the two pooled human liver microsomal preparations.

The rates of ETT metabolism to DETT were correlated with data for total P450 content and a range of P450 form enzyme activities. A good correlation ($r^2 = 0.932$) was observed between ETT metabolism to DETT and CYP3A4-catalyzed testosterone 6β-hydroxylation. Although some correlation ($r^2 = 0.541$) was observed between ETT metabolism to DETT and total P450 content, only low correlations were observed ($r^2 = 0.000$–$0.361$) with P450 form enzyme markers for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP4A9/11. These enzyme activities (with $r^2$ values in parentheses) comprised 7-ethoxyresorufin O-deethylase (0.023, CYP1A2), coumarin 7-hydroxylase (0.022, CYP2A6), $S$-mephenytoin $N$-demethylase (0.310, CYP2B6), paclitaxel 6α-hydroxylase (0.361, CYP2C8), diclofenac 4'-hydroxylase (0.104, CYP2C9), S-mephenytoin 4'-hydroxylase (0.145, CYP2C19), dextromethorphan O-demethylase (0.098, CYP2D6), chlorzoxazone 6-hydroxylase (0.004, CYP2E1), and lauric acid 12-hydroxylase (0.000, CYP4A9/11).

**Metabolism of ETT by Recombinant P450 Forms.** The metabolism of 60 $\mu$M ETT to DETT was examined with rCYP1A2, rCYP2A6, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C19, rCYP2D6, rCYP2E1, and rCYP3A4, together with microsomes from control cells (which contain native CYP1A1) was studied. Incubations were performed for 5 and 30 min. Only trace amounts of DETT were observed in incubations with the control cell microsomes (data not shown) and with rCYP2A6, rCYP2B6, and rCYP2E1 preparations (Fig. 2). Low rates of DETT formation were observed with the rCYP1A2 and rCYP2C8.
preparations, with higher rates being observed with rCYP2C9 and rCYP2C19 preparations. The highest rates of ETT metabolism to DETT were observed with the rCYP2D6 and rCYP3A4 preparations (Fig. 2).

Inhibition of ETT Metabolism. The effect of some human P450 form inhibitors and one P450 form substrate (S-mephenytin) on the metabolism of ETT to DETT in human liver microsomes was studied. For the mechanism-based inhibitors (Newton et al., 1995) furafylline (CYP1A2), diethyldithiocarbamate (CYP2E1), erythromycin (CYP3A4), and tolbutamide (CYP2C9), the compounds were preincubated for 30 min at 37°C with liver microsomes and NADPH prior to the addition of ETT and another aliquot of NADPH. In the studies with the inhibitors sulfaphenazole (CYP2C9) and quinidine (CYP2D6), and the substrate S-mephenytin (CYP2C19), the compounds were preincubated with liver microsome preparations for 10 min at 37°C prior to the addition of NADPH and ETT.

ETT metabolism to DETT was not markedly affected by 5 to 50 μM furafylline, 2 to 50 μM sulfaphenazole, 50 to 500 μM S-mephenytin, and 2 to 20 μM quinidine (Fig. 3, A and B). Whereas 100 μM diethyldithiocarbamate produced some inhibition of ETT metabolism, little effect was observed with lower concentrations of this inhibitor (Fig. 3A).

The effect of erythromycin, miconazole, and tolbutamide on ETT metabolism to DETT in human liver microsomes was studied with both pools of human liver microsomes. ETT metabolism to DETT was markedly inhibited to 3% of control (mean of both liver microsome pools) by 2 to 50 μM erythromycin (CYP3A4), and tolbutamide (CYP2C9), the compounds were preincubated with liver microsomes for 10 min at 37°C with liver microsomes and NADPH (Table 1). When ETT was evaluated as a mechanism-based inhibitor of tolbutamide methylhydroxylase activity, an observed IC50 value (mean of two microsome pools) of 95 μM was obtained. Using a 30-min preincubation period with NADPH, 20 μM tolbutamide (positive control) inhibited tolbutamide methylhydroxylase activity to 18% of control levels.

Brain Penetration of the 5-HT1B/1D Agonists in Mdr1a+/- and Mdr1a++ CF-1 mice. The brain penetration of ETT, NARA, RIZA, SUMA, and ZOLM was investigated in Mdr1a+/- and Mdr1a++/ mice (Umbenhauer et al., 1997). Of the 5-HT1B/1D agonists investigated, ETT was shown to be the best substrate for P-gp; the brain/blood AUC ratio was 13 in Mdr1a+/+ mice, which lack the brain endothelial Mdr1a P-gp efflux pump, and 0.3 in Mdr1a++/ mice, which express Mdr1a P-gp. The mouse P-gp efflux pump therefore has the effect of reducing brain exposure to ETT by approximately 40-fold (Fig. 6A). The effect for all the other triptan molecules, NARA, RIZA, SUMA, and ZOLM, was smaller and less that 5-fold. The brain/blood AUC ratio for inulin, a poorly brain-permeant reference compound, was approximately 0.16 in both mutant and wild-type CF-1 mice, indicating that the integrity of the blood-brain barrier was not compromised by the absence of P-gp. The brain penetration of 5-HT1B/1D agonists in Mdr1a−/− mice appeared to be in line with their lipophlicity (Fig. 6B).

Human MDR1 and Mouse Mdr1a P-Glycoprotein Bidirectional Transport Assay. The transport of NARA, RIZA, SUMA, and ZOLM across LLC-PK1 parent cell monolayers was poor, with <5% of each drug being transported in either the B to A or A to B direction over a 4-h period. This diffusion rate was too low to reliably test whether these compounds were substrates for P-gp. In contrast, the transport of ETT was >10% in this parent cell line, a value consistent with the extent of transport observed for the positive control compounds used: dexamethasone, ritonavir, and verapamil (Table 2). In transport experiments across LLC-MDR1 and LLC-Mdr1a cell monolayers, the BA/AB ratio for ETT was in the range 9 to 11, highlighting it to be a good substrate of human and mouse P-gp in vitro, and similar to the positive control compounds studied.

Discussion

The role of CYP3A and human P-gp in the metabolism and transport of ETT in vivo has been investigated. These studies were performed to understand the potential for ETT to be involved in drug-drug interactions with other drugs that inhibit or induce these

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<td>CYP1A2</td>
<td>7-Ethoxresorufin O-deethylase</td>
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<td>40 (38, 42)</td>
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a Enzyme activity in the absence of added ETT.

b Incubations were performed with the pool II human liver microsomal preparation, except for testosterone 6β-hydroxylase, where both the pool I and II human liver microsomal preparations were used.

c The effect of ETT on the P450 enzyme activities was determined over a concentration range of up to 100 μM.
inhibitors troleandomycin and erythromycin, together with miconazole, produced a marked concentration-dependent inhibition of ETT metabolism to DETT in human liver microsomes (Fig. 4). Previous studies have demonstrated that troleandomycin is a specific inhibitor of human hepatic CYP3A4 (Newton et al., 1995). In addition, both erythromycin and miconazole are also known to inhibit CYP3A4-catalyzed reactions in human liver microsomes (Thummel and Wilkinson, 1998).

Prototypical inhibitors of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP2E (Newton et al., 1995; Ono et al., 1996; Rodrigues, 1999) had little effect on the metabolism of ETT to DETT in human liver microsomes (Fig. 3). Although DETT formation was inhibited by high concentrations of diethylthiocarbamate, this is unlikely to reflect metabolism by CYP2E but, rather, that this compound also inhibits other P450 forms (Ono et al., 1996).

The NADPH-dependent metabolism of 60 µM ETT to DETT by recombinant P450 forms was also studied. The highest rates of DETT formation were observed with rCYP2D6 and rCYP3A4 preparations (Fig. 2). Although ETT was also metabolized to DETT by rCYP2C9 and rCYP2C19 preparations, rates of DETT formation were either much lower or essentially undetectable with the rCYP1A2, rCYP2A6, rCYP2B6, rCYP2C8, and rCYP2E1 preparations.

The observation that ETT is extensively metabolized to DETT by rCYP3A4 is in agreement with the results of the correlation analysis, chemical inhibition, and inhibitory antibody studies. The finding that ETT is also extensively metabolized by rCYP2D6 appears to have little significance when other competing enzymes are present. This finding is borne out by the correlation analysis study, and the lack of effect of quinidine and the CYP2D6-inhibitory antibody on DETT formation in liver microsomes. This result is probably attributable to the relatively higher levels of CYP3A4 compared with those of CYP2D6 in human liver. Levels of CYP3A4/CYP3A4 in human liver microsomes have been reported to range from 44 to 250 pmol/mg protein, whereas those for CYP2D6 have been reported to range from only 5 to 25 pmol/mg protein (Shimada et al., 1994; Rodrigues, 1999).

Many examples of drug-drug interactions in human subjects have been described, and these may be due to either the induction or inhibition of P450-dependent and other xenobiotic metabolizing enzyme activities (Lin and Lu, 1998). The implication of our in vitro data, that of ETT dependence on CYP3A4 for metabolic clearances and its potential for drug interactions when coadministered with CYP3A4 inhibitors or inducers, is that plasma concentrations of ETT in patients would be expected to either increase or decrease, respectively. With regard to coadministration of ETT (Relpax) with CYP3A4 inhibitors, this is borne out in the Product Information for Eletriptan [Pfizer, Sandwich, UK (2001)]. In clinical studies with erythromycin (1000 mg) and ketoconazole (400 mg), which are both strong inhibitors of CYP3A4, significant increases in ETT metabolism to DETT in human liver microsomes (Fig. 3). Although DETT formation was inhibited by high concentrations of diethylthiocarbamate, this is unlikely to reflect metabolism by CYP2E but, rather, that this compound also inhibits other P450 forms (Ono et al., 1996).

Protopypical inhibitors of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP2E1 (Newton et al., 1995; Ono et al., 1996; Rodrigues, 1999) had little effect on the metabolism of ETT to DETT in human liver microsomes (Fig. 3). Although DETT formation was inhibited by high concentrations of diethylthiocarbamate, this is unlikely to reflect metabolism by CYP2E but, rather, that this compound also inhibits other P450 forms (Ono et al., 1996).
CYP2C19, CYP2D6, CYP2E1, and CYP3A4 studied (Table 1). These data suggest that ETT is unlikely to produce any significant inhibitory interactions in vivo with other drugs that are metabolized by these P450 forms.

P-gp plays a role in modulating oral exposure and brain penetration. Unlike the other triptans investigated, ETT was an excellent substrate for human and mouse P-gp in vitro, with BA/AB ratios in bidirectional transport studies across LLC-MDR1 and LLC-Mdr1a cell monolayers in the range 9 to 11 (Table 2). This finding has significance clinically since it was reported recently that administration of both ETT and verapamil resulted in a 2.7- and a 2.2-fold increase in ETT AUC and C_{max} respectively (Humphrey, 2002). These data also have significance preclinically, since brain exposure to ETT was reduced 40-fold in Mdr1a^{-/-} relative to Mdr1a^{+/-} mice (Fig. 6A).

The finding that ETT is a good mouse and human P-gp substrate led us to investigate four other triptans in these models. This was undertaken to enable us to speculate on the role of P-gp in modulating a central mechanism of action for the triptan class of antimigraine drug. Three potential mechanisms of action have been proposed for 5-HT_{1B/1D} receptor-selective agonists in the treatment of acute migraine attacks. The first two invoke peripheral mechanisms: vasoconstriction of dilated, pain-producing meningeal blood vessels and inhibition of meningeal perivascular trigeminal nerves, which reduces the release of, for example, the powerful vasodilator calcitonin gene-related peptide. The third mechanism, for brain-penetrant compounds, is antinociceptive activity within the brain stem sensory trigeminal nuclei; anatomical studies having shown a high concentration of 5-HT_{1B/1D} receptors in the trigeminal ganglion of animals and humans. It might be that each of these mechanisms operates in a complementary manner to reduce the intense central trigeminal input that occurs during a migraine headache (Goadsby and Hargreaves, 2000). If the central site is important for antimigraine efficacy, then P-gp might be expected to limit the accessibility of ETT to central sites of action. An analysis of the available pharmacokinetic data for lead triptans indeed indicates that higher free drug levels of ETT, the most potent of all the triptans at 5HT_{1B/1D} receptors in vitro, are increased potency and lipophilicity on improved efficacy and barrier transport (gut and brain) for ETT may, therefore, have been effectively offset by relatively high plasma protein binding and good substrate specificity for P-glycoprotein (Fig. 6B, Table 3).

It is recognized that the triptan class of compounds do generally have poor brain penetration characteristics when compared with typical CNS marketed drugs (Doan et al., 2002). The extent of brain

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**Table 3**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>BA/AB Ratio</th>
<th>% Transport at t = 4 h</th>
<th>BA/AB Ratio</th>
<th>% Transport at t = 4 h</th>
<th>BA/AB Ratio</th>
<th>% Transport at t = 4 h</th>
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<td></td>
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<td>B to A</td>
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<tr>
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<td>1.6</td>
<td>3.0</td>
<td>2.7</td>
<td>1.2</td>
<td>4.2</td>
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<tr>
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<td>2.5</td>
<td>2.2</td>
<td>1.4</td>
<td>2.9</td>
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<td>14</td>
<td>18</td>
<td>4.3</td>
<td>6.1</td>
<td>26</td>
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<tr>
<td>Ritonavir</td>
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<td>18</td>
<td>24</td>
<td>27</td>
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<tr>
<td>Verapamil</td>
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<td>24</td>
<td>4.9</td>
<td>5.4</td>
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**Table 2**

Bidirectional transport of 5-HT_{1B/1D} agonists across LLC-PK1, LLC-MDR1, and LLC-Mdr1a cell monolayers

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LLC-PK1</th>
<th>LLC-MDR1</th>
<th>LLC-Mdr1a</th>
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<tr>
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<td>BA/AB Ratio</td>
<td>% Transport at t = 4 h</td>
<td>BA/AB Ratio</td>
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<td>A to B</td>
<td>B to A</td>
<td>A to B</td>
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<tr>
<td>ETT80</td>
<td>382</td>
<td>0.24</td>
<td>7.7 ± 0.02</td>
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<tr>
<td>ZOLM5</td>
<td>287</td>
<td>0.75</td>
<td>7.6 ± 0.05</td>
</tr>
<tr>
<td>RIZA10</td>
<td>269</td>
<td>0.86</td>
<td>7.1 ± 0.04</td>
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<tr>
<td>SUMA100</td>
<td>295</td>
<td>0.82</td>
<td>7.0 ± 0.03</td>
</tr>
<tr>
<td>ETT40</td>
<td>382</td>
<td>0.24</td>
<td>7.7 ± 0.02</td>
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</tbody>
</table>

---

**Table 1**

Physicochemical, pharmacological, and pharmacokinetic properties of selected 5-HT_{1B/1D} agonists

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg)</th>
<th>f_{u}</th>
<th>5-HT_{1B} Potency</th>
<th>5-HT_{1D} Potency</th>
<th>Human Pharmacokinetic Parameters</th>
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<tr>
<td></td>
<td>mol. wt.</td>
<td></td>
<td>pIC50</td>
<td>pIC50</td>
<td>C_{max}</td>
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<td>ETT80</td>
<td>382</td>
<td>0.24</td>
<td>7.7 ± 0.02</td>
<td>9.2 ± 0.02</td>
<td>226</td>
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<tr>
<td>ZOLM5</td>
<td>287</td>
<td>0.75</td>
<td>7.6 ± 0.05</td>
<td>8.9 ± 0.06</td>
<td>73.9-1</td>
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<td>RIZA10</td>
<td>269</td>
<td>0.86</td>
<td>7.1 ± 0.04</td>
<td>8.4 ± 0.05</td>
<td>19.8</td>
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<tr>
<td>Doses that provide equivalent efficacy to SUMA100</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUMA100</td>
<td>295</td>
<td>0.82</td>
<td>7.0 ± 0.03</td>
<td>8.3 ± 0.05</td>
<td>54-78</td>
</tr>
<tr>
<td>ETT40</td>
<td>382</td>
<td>0.24</td>
<td>7.7 ± 0.02</td>
<td>9.2 ± 0.02</td>
<td>113</td>
</tr>
</tbody>
</table>

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* Substrate concentrations were 10 μM for ETT, NARA, RIZA, and ZOLM, 0.5 μM for verapamil, and 5 μM for ritonavir and dexamethasone.
* Analyses were performed in triplicate and mean data are expressed to two significant figures (percentage CVs were <10% for ETT and <30% for NARA, RIZA, SUMA, and ZOLM).
penetration is, however, a poor guide to central activity, especially with potent agonist drugs such as the triptans, since they, in contrast to most other CNS agents that are antagonists, will require only low fractional receptor occupancy to exert central effects. Further studies are warranted to relate plasma and brain concentrations of the triptans to their occupancy of central antimigraine 5-HT1B/1D receptors in vivo to evaluate the potential contribution of central sites to their mechanism of action.

In summary, the results of this study demonstrate that ETT can be metabolized to DETT by human liver microsomal preparations, and this biotransformation is primarily catalyzed by CYP3A4. ETT was determined not to be a potent inhibitor of a number of P450-dependent enzyme activities, although ETT metabolism was markedly reduced in vitro by prototypical CYP3A4 inhibitors. These findings are in agreement with the clinical data inasmuch as plasma levels of ETT are increased in human subjects when ETT is coadministered with inhibitors of CYP3A. ETT was shown to be a substrate of human P-glycoprotein, although the importance of this in the context of a central mechanism of action remains inconclusive.

Acknowledgments. We thank P. Scott-Stevens (Department of Medicinal Chemistry, Merck Sharp & Dohme, Terlings Park, Harlow, Essex, UK), L. Romanyshyn (Drug Metabolism, Merck & Co., Rahway, NJ), R. J. Price, A. B. Renwick, P. J. Young, and D. G. Walters (BBIRA International Ltd., Surrey, UK) for contributions to the manuscript.

References


