

ELETRIPTAN METABOLISM BY HUMAN HEPATIC CYP450 ENZYMES AND TRANSPORT BY HUMAN P-GLYCOPROTEIN

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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 ($r^2 = 0.932$) was obtained between formation of *N*-desmethyl eletriptan (DETT) and CYP3A4-catalyzed testosterone 6 β -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 by 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 troleandomycin, 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^{+/+} 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]-1*H*-indole] (Fig. 1) is a selective agonist at 5-hydroxytryptamine (serotonin)_{1B/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

¹ Abbreviations used are: ETT, eletriptan; DETT, *N*-desmethyl eletriptan; P-gp, P-glycoprotein; NARA, naratriptan; RIZA, rizatriptan; SUMA, sumatriptan; ZOLM, zolmitriptan; LC/MS-MS, liquid chromatography/tandem mass spectrometry; HPLC, high-pressure liquid chromatography; BA/AB, basolateral-to-apical/apical-to-basolateral transport ratio; 5-HT, 5-hydroxytryptamine (serotonin); P450, cytochrome P450; AUC, area under the curve.

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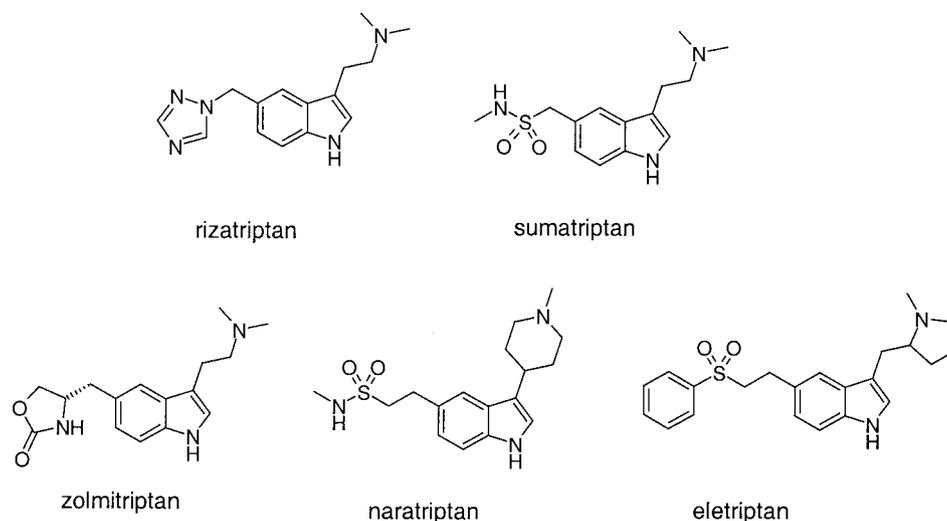


Fig. 1. Structures of 5-HT_{1B/1D} agonists.

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, quinidine, sulfaphenazole, troleandomycin 7-ethoxyresorufin, resorufin, tolbutamide, debrisoquine, chlorzoxazone, testosterone, and 6 β -hydroxytestosterone were obtained from Sigma-Aldrich Company Ltd. (Poole, Dorset, UK); and furafylline, *S*(+)-mephenytoin, methylhydroxytolbutamide, 4'-hydroxymephenytoin, *N*-desmethyl *S*-mephenytoin (Nirvanol), 4-hydroxydebrisoquine, and 6-hydroxychlorzoxazone were obtained from Salford Ultrafine Chemicals and Research Ltd. (Manchester, UK). [*Guanidine*-¹⁴C]debrisoquine, *S*-[4-¹⁴C]mephenytoin, and [4-¹⁴C]testosterone (specific activities 53, 56, and 56 mCi/mmol, respectively) were purchased from Amersham Biosciences UK Ltd. (Little Chalfont, Buckinghamshire, UK). A reaction phenotyping kit (Product Number H0500, Version 5) containing 16 individual human liver microsomal preparations, characterized for total P450 content and a range of P450 isoform enzyme activities, was purchased from Xenotech LLC (Kansas City, KS) and stored at -80°C . Microsomes from human B-lymphoblastoid cells containing cDNA-expressed human P450 isoforms (BD Gentest, Woburn, MA) were obtained from Cambridge Bioscience (Cambridge, UK) and stored at -80°C . The samples of human B-lymphoblastoid cell microsomes comprised control cell microsomes (i.e., no transfected human cytochrome P-450 isoform cDNA but contains native CYP1A1 activity) and cell microsomes containing CYP1A2, CYP2A6 + OR [i.e., CYP2A6 cDNA plus human NADPH-cytochrome P-450 reductase (OR) cDNA], CYP2B6, CYP2C8 + OR, CYP2C9 + OR, CYP2C19, CYP2D6 + OR, CYP2E1 + OR, and CYP3A4 + OR. Neither CYP3A5 nor the effect of cytochrome *b*₅ on P450 activities was investigated in our studies. Inhibitory antibodies against CYP2C8/9, CYP2D6, and CYP3A4 were provided by Merck Research Laboratories (Rahway, NJ) and stored at -80°C . The specificity of these inhibitory antibodies has been demonstrated for CYP3A4 and CYP2D6 (Mei et al., 2002), and for CYP2C8/9, also based on in-house studies (unpublished data).

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 incubation mixtures 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 HTS 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 \times 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 \rightarrow 84, DETT *m/z* 369 \rightarrow 70, and L-844151 internal standard *m/z* 354 \rightarrow 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

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 MgCl_2 , 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 MgCl_2 , 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) 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 MgCl_2 , 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 processed for LC/MS-MS analysis as described above.

To study the effect of inhibitory antibodies to CYP2C8/9, CYP2D6, and CYP3A4 forms, incubation mixtures contained 10 mM MgCl_2 , 2 mM EDTA, 84 mM phosphate buffer, pH 7.4, 0.1 mg of microsomal protein, and either 0.025, 0.25, or 2.5 μl of the antibody preparation in a final volume of 0.5 ml. After a 10-min preincubation at 37°C in a shaking water bath, the reaction was initiated by the addition of 60 μM ETT and 1 mM NADPH (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.

Effect of ETT on CYP450 Enzyme Activities. The activities of 7-ethoxyresorufin *O*-deethylase, tolbutamide methylhydroxylase, debrisoquine 4-hydroxylase, and testosterone 6 β -hydroxylase were determined as described previously (Renwick et al., 2000), employing substrate concentrations of 0.2, 180, 150, and 50 μM , respectively. For tolbutamide and debrisoquine metabolism, the tubes were preincubated in duplicate at 37°C in a shaking water bath for 5 min in the presence of either no ETT (controls) or 0.2 to 100 μM ETT and an NADPH-generating system, prior to addition of the P450 substrate. The effect of ETT on 7-ethoxyresorufin metabolism was determined as described for tolbutamide and debrisoquine metabolism, but with an ETT concentration range of 0.5 to 100 μM . For testosterone 6 β -hydroxylase, the tubes were preincubated in duplicate at 37°C in a shaking water bath for 5 min in the presence of either no ETT (controls) or ETT (concentration range 0.2–100 μM or 1–100 μM) and 50 μM [4-¹⁴C]testosterone (0.5 μCi /tube), prior to the addition of 1 mM NADPH. To evaluate the effect of ETT as a mechanism-

based inhibitor of testosterone 6 β -hydroxylase activity, the tubes were preincubated at 37°C in a shaking water bath for 30 min in the presence of either no ETT (controls) or ETT (concentration range 0.5–50 μM or 0.2–100 μM) and 1 mM NADPH, prior to the addition of 50 μM [4-¹⁴C]testosterone (0.5 μCi /tube) and 1 mM NADPH (final concentration 2 mM). Chlorzoxazone 6-hydroxylase was determined in incubation mixtures containing 45 μM chlorzoxazone, 1 mM NADPH, 0.2 mg of microsomal protein, and 0.1 M phosphate buffer in a final volume of 1 ml. Incubations were terminated after 20 min with 0.2 ml of 30% (v/v) perchloric acid, and the supernatant was analyzed by HPLC. Chromatography was performed as described previously for tolbutamide methylhydroxylase (Renwick et al., 2000), except that the mobile phase consisted of 23% (v/v) acetonitrile and 77% (v/v) 20 mM sodium perchlorate, pH 2.5, and the eluant was monitored at 287 nm. The metabolism of *S*-mephenytoin to both 4'-hydroxymephenytoin and *N*-desmethyl *S*-mephenytoin was determined in incubation mixtures containing 60 μM *S*-[4-¹⁴C]mephenytoin (0.5 μCi /tube), 1 mM NADPH, 7.5 mM DL-isocitric acid, 2 U/ml isocitric dehydrogenase, 5 mM MgSO_4 , 0.5 mg microsomal protein and 0.1 M phosphate buffer pH 7.4 in a final volume of 0.5 ml. Incubations were terminated after 30 min with 0.1 ml of 30% (v/v) perchloric acid and the supernatant analyzed by HPLC. Chromatography was performed as described previously for debrisoquine 4-hydroxylase (Renwick et al., 2000), except that the mobile phase consisted of 17% (v/v) acetonitrile and 83% (v/v) 20 mM sodium perchlorate, pH 2.5, with quantitation by liquid scintillation counting. The effect of 0.2 to 100 μM ETT on chlorzoxazone and *S*-mephenytoin metabolism was studied using a preincubation period of 5 min as described above for tolbutamide and debrisoquine. For all P450 form enzyme activities studied, plots were constructed of enzyme activity (percentage of control rate in the absence of added ETT) against ETT concentration (logarithmic scale). Where inhibition of enzyme activity was observed, IC_{50} values (i.e., concentration of ETT to produce a 50% inhibition of enzyme activity) were calculated by linear regression analysis.

Brain Penetration of the 5-HT_{1B/1D} Agonists in Mdr1a^{-/-} and Mdr1a^{+/+} CF-1 Mice. The impact of the drug efflux pump, P-gp (Umbenhauer et al., 1997) on the brain entry of the triptan drugs was investigated in vivo using male CF-1 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 CF-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 (–20°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 μl of a 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 KR100-5C8 HPLC column (150 \times 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, Bailer's method for determining area under

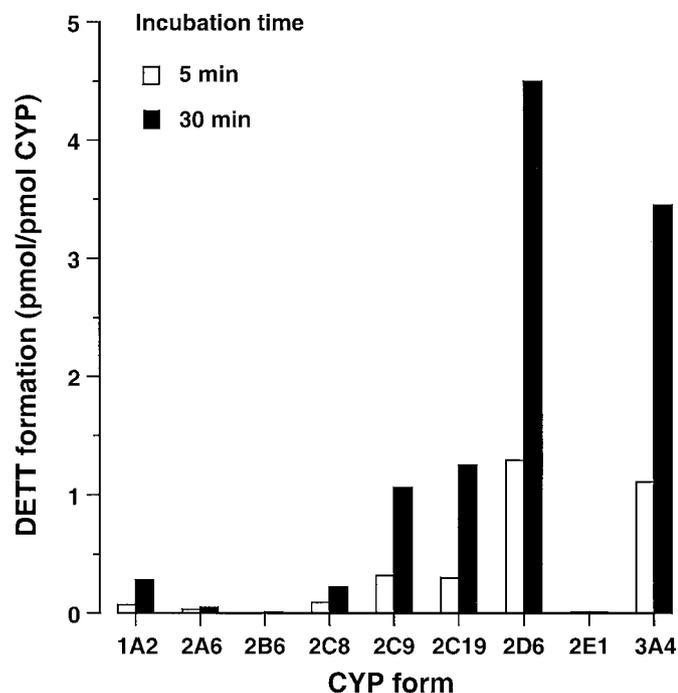


FIG. 2. Metabolism of ETT to DETT by recombinant human P450 forms.

Incubations were performed with 60 μM ETT and 0.1 mg of B-lymphoblastoid cell microsomal protein for either 5 or 30 min. Results are presented as the mean of a duplicate test incubation.

the curve (AUC) confidence intervals during sparse sampling was applied (Bailer, 1988; Nedelman et al., 1995). Standard error of the mean (S.E.M.) for the brain/blood ratio was determined using the following equation: $\text{S.E.M.} = (\text{Brain AUC S.E.M.}^2 / \text{Blood AUC}^2) + (\text{Brain AUC}^2 \cdot \text{Blood AUC S.E.M.}^2 / \text{Blood AUC}^4)$. An unpaired two-tailed t test was performed to determine the significance of the difference between brain/blood $\text{AUC}_{0.5-6\text{h}}$ ratios determined in $\text{Mdr1a}^{-/-}$ and $\text{Mdr1a}^{+/+}$ 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 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 cultured in 24-well Transwell plates (Falcon) at a density of 1.5×10^5 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 ritonavir, 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 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 used was 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

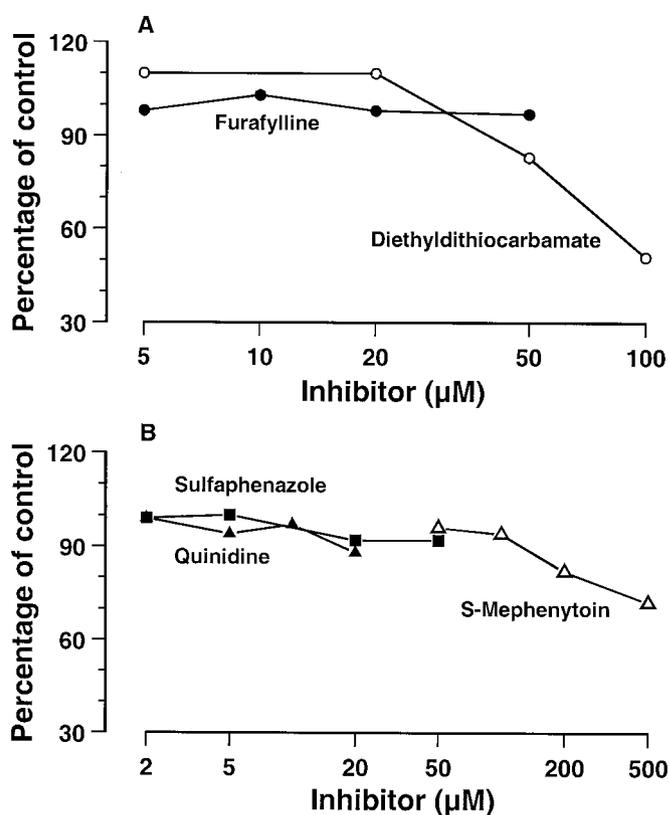


FIG. 3. Inhibition of ETT metabolism to DETT in human liver microsomes.

The effects of the mechanism-based inhibitors 5 to 50 μM furafylline (●) and 5 to 100 μM diethyldithiocarbamate (○) (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.

solution concentration for an 11-point standard curve. Percentage of transport was calculated by dividing the concentration of the compound appearing in the receiver compartment by the sum of the compound concentrations measured in the receiver and donor compartment ($\times 100\%$). 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 well) by the concentration of compound measured in the basolateral compartment (resulting from introduction of the compound in the apical well).

Lipophilicity Measurements. Octanol-pH 7.4 buffer partition coefficients ($\log D_{7.4}$) 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 both 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

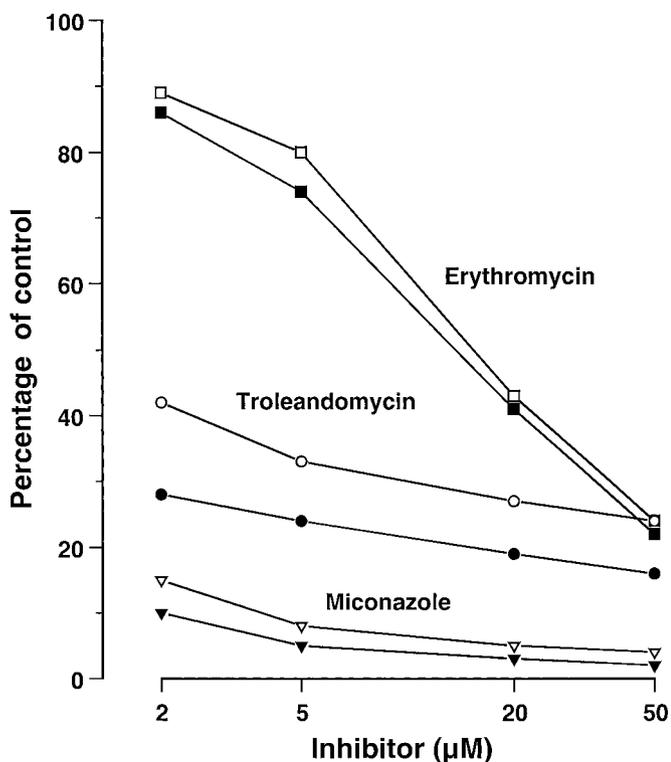


Fig. 4. Effect of CYP3A inhibitors on ETT metabolism to DETT in human liver microsomes

The effects of 2 to 50 μM concentrations of the mechanism-based inhibitors troleandomycin (●, ○) and erythromycin (■, □), together with 2 to 50 μM miconazole (▼, ▽) were evaluated on the metabolism of 60 μM ETT to DETT. Incubations were performed with 0.1 mg protein of either the pool I (filled symbols) or pool II (open symbols) human liver microsomal preparations for 5 min. Each point represents the mean of a duplicate test incubation.

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 μM (pool I and pool II individual values were 24.3 and 29.9 μM , 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 $\mu\text{l}/\text{min}/\text{mg}$ protein (pool I and pool II individual values were 4.34 and 1.69 $\mu\text{l}/\text{min}/\text{mg}$ protein).

An ETT substrate concentration of 60 μM 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 μM 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.

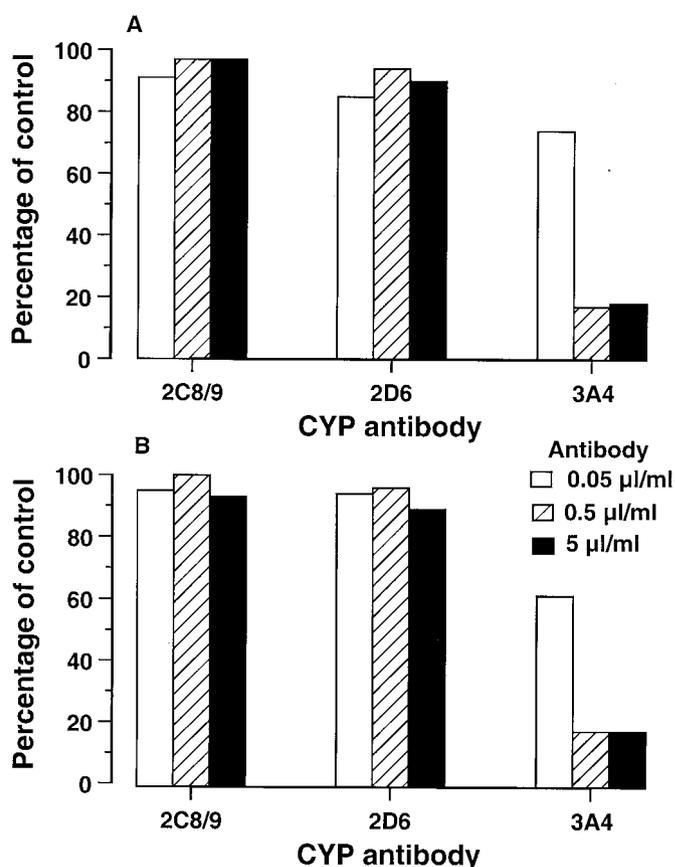


Fig. 5. Effect of inhibitory antibodies to CYP2C8/9, CYP2D6, and CYP3A4 on ETT metabolism to DETT in human liver microsomes.

Incubations were performed with 60 μM ETT and 0.1 mg protein of the pool I (A) and pool II (B) human liver microsomal preparations in the presence of 0, 0.05, 0.5, and 5 $\mu\text{l}/\text{ml}$ incubation of the three P450 form antibodies for 5 min. Results are presented as the mean of a duplicate test incubation.

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 6 β -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 enzymatic 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 6 α -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 μM ETT to DETT by 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

TABLE 1
Effect of ETT on some P450 enzyme activities in human liver microsomes

P450	Enzyme Activity	Control Rate ^{a,b}	ETT IC ₅₀ ^c
		pmol/min/mg protein	μM
CYP1A2	7-Ethoxyresorufin <i>O</i> -deethylase	39	>100
CYP2B6	<i>S</i> -Mephenytoin <i>N</i> -demethylase	14	>100
CYP2C9	Tolbutamide methylhydroxylase	98	>100
CYP2C19	<i>S</i> -Mephenytoin 4'-hydroxylase	4.1	>100
CYP2D6	Debrisoquine 4-hydroxylase	60	84
CYP2E1	Chlorzoxazone 6-hydroxylase	400	>100
CYP3A4	Testosterone 6β-hydroxylase	819 (836, 802)	95 (87, 100)
CYP3A4	Testosterone 6β-hydroxylase (mechanism-based inhibition)	723 (760, 685)	40 (38, 42)

^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.

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*-mephenytoin) on the metabolism of ETT to DETT in human liver microsomes was studied. For the mechanism-based inhibitors (Newton et al., 1995) furafylline (CYP1A2), diethylthiocarbamate (CYP2E1), erythromycin (CYP3A4), and troleandomycin (CYP3A4), 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*-mephenytoin (CYP2C19), the compounds were preincubated with liver microsomes 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*-mephenytoin, and 2 to 20 μM quinidine (Fig. 3, A and B). Whereas 100 μM diethylthiocarbamate produced some inhibition of ETT metabolism, little effect was observed with lower concentrations of this inhibitor (Fig. 3A).

The effect of erythromycin, miconazole, and troleandomycin on ETT metabolism to DETT was studied with both pools of human liver microsomes. ETT metabolism to DETT was markedly inhibited to 3 to 13% of control (mean of both liver microsome pools) by 2 to 50 μM miconazole and to 20 to 35% of control by 2 to 50 μM troleandomycin (Fig. 4). Erythromycin also produced a concentration-dependent inhibition of ETT metabolism, with enzyme activity being reduced to 23% of control by 50 μM erythromycin.

The effect of inhibitory antibodies to CYP2C8/9, CYP2D6 and CYP3A4 on ETT metabolism to DETT in human liver microsomes was studied. With both pools of human liver microsomes treatment with 0.05–5 μl/ml incubation of either the CYP2C8/9 or the CYP2D6 antibodies had little effect on ETT metabolism (Fig. 5A and B). In contrast, the CYP3A4 antibody produced a marked inhibition of the metabolism of ETT to DETT in human liver microsomes.

Effect of ETT on P450 Form Enzyme Activities. The effect of addition of ETT at concentrations of up to 100 μM on some P450 form enzyme activities in human liver microsomes was studied. ETT produced a weak inhibition of CYP2D6-dependent debrisoquine 4-hydroxylase activity, with a calculated IC₅₀ value (i.e., concentration of ETT to produce a 50% inhibition of enzyme activity) of 84 μM (Table 1). The addition of ETT had no effect (IC₅₀ values all >100 μM) on 7-ethoxyresorufin *O*-deethylase (CYP1A2), *S*-mephenytoin *N*-demethylase (CYP2B6), tolbutamide methylhydroxylase (CYP2C9), *S*-

mephenytoin 4'-hydroxylase (CYP2C19), and chlorzoxazone 6-hydroxylase (CYP2E1) activities. ETT produced a weak inhibition of CYP3A4-dependent testosterone 6β-hydroxylase activity, with a calculated IC₅₀ value (mean of two liver microsome pools) of 95 μM (Table 1). When ETT was evaluated as a mechanism-based inhibitor of testosterone 6β-hydroxylase activity, an observed IC₅₀ value (mean of two liver microsome pools) of 40 μM was obtained. Using a 30-min preincubation period with NADPH, 20 μM troleandomycin (positive control) inhibited testosterone 6β-hydroxylase activity to 18% of control levels.

Brain Penetration of the 5-HT_{1B/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 (Umberhauer et al., 1997). Of the 5-HT_{1B/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 than 5-fold. The brain/blood AUC ratio for inulin, a poorly brain-penetrant 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-HT_{1B/1D} agonists in Mdr1a^{-/-} mice appeared to be in line with their lipophilicity (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 vitro 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

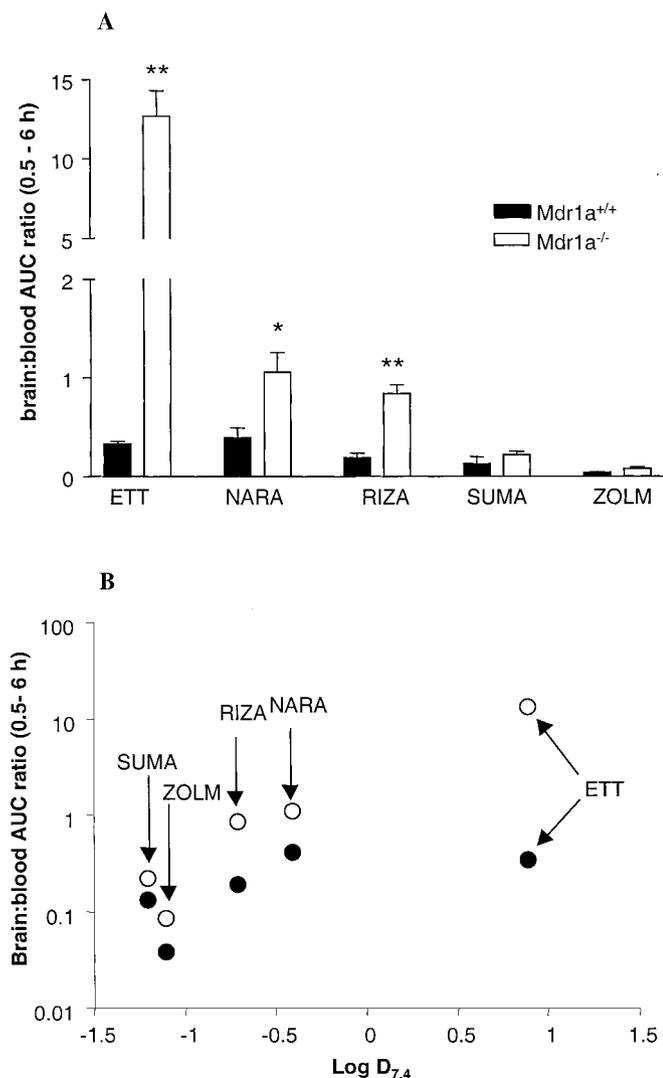


Fig. 6. Brain penetration of 5-HT_{1B/1D} agonists in CF-1 Mdr1a^{+/+} and Mdr1a^{-/-} mice (A) and in CF-1 mice versus lipophilicity (B).

A, brain/blood AUC_{0.5-6h} ratios for 5-HT_{1B/1D} agonists after intravenous administration to Mdr1a^{+/+} and Mdr1a^{-/-} CF-1 mice. Error bars are S.E.M. (three mice per time point, four time points). **, $p < 0.01$; *, $p < 0.05$. B, open circles are brain/blood ratio data in Mdr1a^{-/-} mice; closed circles are brain/blood ratio data in Mdr1a^{+/+} mice.

proteins. These are the first detailed studies of this kind to be reported for ETT. In addition, since a central mode of action for RIZA, NARA, and ZOLM has been proposed (Goadsby, 2000; Goadsby and Hargreaves, 2000; Goadsby et al., 2002), factors that regulate the brain penetration of ETT, relative to NARA, RIZA, SUMA, and ZOLM, were investigated also and included measurements of lipophilicity and brain penetration in CF-1 wild-type (Mdr1a^{+/+}) and P-gp-deficient (Mdr1a^{-/-}) mice.

The NADPH-dependent *N*-demethylation of ETT to DETT was investigated in two preparations of pooled human liver microsomes and in 16 individual liver microsomes preparations, and was concluded to be mediated by CYP3A, a good correlation ($r^2 = 0.932$) being observed between DETT formation and testosterone 6 β -hydroxylase activity. The effect of erythromycin, miconazole, and troleanomycin on ETT metabolism to DETT was also studied with both preparations of pooled human liver microsomes. No significant differences were noted between the two human liver microsomes pools in the effects of the three compounds on DETT formation. The mechanism-based

inhibitors troleanomycin 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 troleanomycin 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 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 CYP2E1 but, rather, that this compound also inhibits other P450 forms (Ono et al., 1996).

The NADPH-dependent metabolism of 60 μ M 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 CYP3A/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 C_{max} (2- and 2.7-fold) and AUC (3.6- and 5.9-fold), respectively, were observed. This increased exposure was associated with an increase in ETT half-life from 4.6 h to 7.1 h for erythromycin and from 4.8 h to 8.3 h for ketoconazole. Coadministration of ETT with CYP3A4 inhibitors is therefore discouraged [Product Information for Eletriptan, Pfizer, Sandwich, UK (2001); Tepper et al., 2003]. No information is provided with regard to coadministration of ETT with strong CYP3A4 inducers such as phenytoin, rifampicin, and others (Smith, 2000), but a reduction in ETT plasma concentrations would be expected following such a dosing regimen.

In our studies, the potential for ETT to produce drug-drug interactions as a consequence of inhibiting P450-mediated reactions was also evaluated. Overall, ETT at concentrations of up to 40 μ M had little effect on the enzymatic markers of CYP1A2, CYP2B6, CYP2C9,

TABLE 2
Bidirectional transport of 5-HT_{1B/1D} agonists across LLC-PK1, LLC-MDR1, and LLC-Mdr1a cell monolayers

Analyte ^a	LLC-PK1			MDR1			Mdr1a		
	BA/AB Ratio	% Transport at <i>t</i> = 4 h ^b		BA/AB Ratio	% Transport at <i>t</i> = 4 h ^b		BA/AB Ratio	% Transport at <i>t</i> = 4 h ^b	
		A to B	B to A		A to B	B to A		A to B	B to A
Eletriptan	1.5	13	23	11	2.5	32	9.1	3.3	38
Naratriptan	1.3	3.1	3.8	3.2	1.2	4.1	2.1	3.4	6.3
Rizatriptan	1.1	4.9	4.9	3.2	2.3	8.2	2.1	2.9	8.6
Sumatriptan	1.5	1.6	3.0	2.7	1.2	4.2	1.1	2.9	3.5
Zolmitriptan	1.0	2.2	2.5	2.2	1.4	2.9	1.2	3.2	3.7
Dexamethasone	1.3	14	18	4.3	6.1	26	4.5	6.1	25
Ritonavir	1.3	18	24	27	1.2	29	11	2.5	25
Verapamil	1.4	18	24	4.9	5.4	33	6.3	5.8	38

^a Substrate concentrations were 10 μM for ETT, NARA, RIZA, SUMA, and ZOLM, 0.5 μM for verapamil, and 5 μM for ritonavir and dexamethasone.

^b 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).

TABLE 3
Physicochemical, pharmacological, and pharmacokinetic properties of selected 5-HT_{1B/1D} agonists

Compound Dose	mol. wt.	<i>f</i> _u ^a	5-HT _{1B} Potency ^b	5-HT _{1D} Potency ^b	Human Pharmacokinetic Parameters ^c			
					Total C _{max}	<i>f</i> _u C _{max}	Total AUC	<i>f</i> _u AUC
					<i>pEC</i> ₅₀	<i>pEC</i> ₅₀	ng/ml	nM
Doses that provide superior efficacy to SUMA100 ^d								
ETT80	382	0.24	7.7 ± 0.02	9.2 ± 0.02	226	142	1486	934
ZOLM5	287	0.75	7.6 ± 0.05	8.9 ± 0.06	7.3–9.1	19–24	52–62	136–162
RIZA10	269	0.86	7.1 ± 0.04	8.4 ± 0.05	19.8	63	49.6	158
Doses that provide equivalent efficacy to SUMA100 ^d								
SUMA100	295	0.82	7.0 ± 0.03	8.3 ± 0.05	54–78	150–216	419	1164
ETT40	382	0.24	7.7 ± 0.02	9.2 ± 0.02	113	71	743	467

^a Plasma protein binding (PPB) for ETT was reported as 76% by Morgan et al. 1997, which was in agreement with an in-house determination of PPB for 200 ng/ml ETT of 75 ± 3%. PPB data for ZOLM, RIZA, and SUMA were abstracted from the product labels [Product Information for Rizatriptan, Merck and Co. Inc., West Point, PA, (1998); Product Information for Sumatriptan; Glaxo Wellcome Inc., Research Triangle Park, NC (1998); Product Information for Zolmitriptan, AstraZeneca Pharmaceuticals LP, Wilmington, DE (1998)]. The reported value of PPB for SUMA was also in agreement with the value determined in-house (17 ± 4%).

^b 5-HT_{1B/1D} potency data were abstracted from Stewart et al. (1999).

^c Pharmacokinetic data were abstracted from Palmer and Spencer (1997) for ZOLM, Sciberras et al. (1997) for RIZA, and Perry and Markham (1998) for SUMA. Pharmacokinetic data for ETT40 and ETT80 were approximated by pro-rating data from an oral dose ranging study that used 15, 20, 30, 60, 90, and 120 mg of ETT (Milton et al., 2002); ETT C_{max} = 2.828 · Dose (*r*² = 0.96), ETT AUC = 18.577 · Dose (*r*² = 0.97).

^d ETT80, ZOLM5, and RIZA10 provided equivalent efficacy and were superior to SUMA100 with respect to percentage of patients free from headache at 2 h and patients with improvements in headache at 2 h (Goadsby et al., 2002). Using these efficacy criteria, ETT40 was equivalent to SUMA100.

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 required for clinical efficacy when compared with ZOLM or RIZA at doses that provide equivalent efficacy (Table 3; Goadsby et al., 2002). These higher plasma levels of ETT may reflect a requirement to provide sufficient free drug levels in the brain for adequate occupancy of central 5-HT_{1B/1D} receptors. The potential theoretical gains of 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

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-HT_{1B/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.

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References

- Bailer AJ (1988) Testing for the equality of area under the curves when using destructive measurement techniques. *J Pharmacol Biopharm* **16**:303–309.
- Cummins CL, Jacobsen W, and Benet LZ (2002) Unmasking the dynamic interplay between intestinal P-glycoprotein and CYP3A4. *J Pharmacol Exp Ther* **300**:1036–1045.
- Doan KMH, Humphreys JE, Webster LO, Wring SA, Shampine LJ, Serabjit-Singh CJ, Adkison KK, and Polli JW (2002) Passive permeability and P-glycoprotein-mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs. *J Pharmacol Exp Ther* **303**:1–9.
- Dresser GK, Schwarz UI, Wilkinson GR, and Kim RB (2003) Coordinate induction of both cytochrome P4503A and MDR1 by St John's wort in healthy subjects. *Clin Pharmacol Ther* **73**:41–50.
- Goadsby PJ (2000) The pharmacology of headache. *Prog Neurobiol* **62**:505–525.
- Goadsby PJ and Hargreaves RJ (2000) Mechanisms of action of serotonin 5-HT_{1B/1D} agonists: insights into migraine pathophysiology using rizatriptan. *Neurology* **55**: S8–S14.
- Goadsby PJ, Lipton RB, and Ferrari MD (2002) Migraine—current understanding and treatment. *N Engl J Med* **346**:257–270.
- Hansch C and Leo A (1979) *Substituent Constants for Correlation Analysis in Chemistry and Biology*. Wiley-Interscience, New York.
- Humphrey MJ (2002) inventor, Pfizer Limited, UK; Pfizer Inc., assignee. Compositions containing eletriptan and p-glycoprotein for improved drug bioavailability. Pfizer Limited, UK; Pfizer, Inc. PCT Int. Appl., 29 pp. CODEN: PIXXD2 WO 0270070 A2 20020912, WO 2002-IB512 20020220. Priority: GB 2001-5131 20010301. CAN 137:222088 AN 2002: 695836. 2002 Sep 12.
- Jackson NC (1996) A comparison of oral eletriptan (UK-116,044) (20–80 mg) and oral sumatriptan (100 mg) in the acute treatment of migraine. *Cephalalgia* **16**:368–369.
- Lin JH and Lu AYH (1998) Inhibition and induction of cytochrome P450 and the clinical implications. *Clin Pharmacokinet* **35**: 361–390.
- Mei Q, Tang C, Lin Y, Rushmore TH, and Shou M (2002) Inhibition kinetics of monoclonal antibodies against cytochromes P450. *Drug Metab Dispos* **30**:701–708.
- Milton KA, Allen MJ, Abel S, Jenkins VC, James GC, Rance DJ, and Eve MD (1997) The safety, tolerability, pharmacokinetics and pharmacodynamics of oral and intravenous eletriptan, a potent and selective "5HT_{1D}-like" receptor partial agonist. *Cephalalgia* **17**:414–415.
- Milton KA, Scott NR, Allen MJ, Abel S, Jenkins VC, James GC, Rance DJ, and Eve MD (2002) Pharmacokinetics, pharmacodynamics and safety of the 5-HT_{1B/1D} agonist eletriptan following intravenous and oral administration. *J Clin Pharmacol* **42**:528–539.
- Morgan P, Mitchell RJ, Webster R, Gedge J, Nedderman A, Macrae PV, and Wright P (2000) The metabolism of [¹⁴C]-eletriptan in rat, dog and human. *Drug Metab Rev* **32** (Suppl 1):83.
- Morgan P, Rance D, James CG, and Milton KA (1997) An *in vitro-in vivo* correlation of eletriptan pharmacokinetics in rat, dog and human. *Headache* **37**:324.
- Nedelman JR, Gibiansky E, and Lau DTW (1995) Applying Bailor's method for AUC confidence intervals to sparse sampling. *Pharm Res (NY)* **12**:124–128.
- Newton DJ, Wang RW, and Lu AYH (1995) Cytochrome P450 inhibitors. Evaluation of specificities in the *in vitro* metabolism of therapeutic agents by human liver microsomes. *Drug Metab Dispos* **23**:154–158.
- Ono S, Hatanaka T, Hotta H, Satoh T, Gonzalez FJ, and Tsutsui M (1996) Specificity of substrate and inhibitor probes for cytochrome P450s: evaluation of *in vitro* metabolism using cDNA-expressed human P450s and human liver microsomes. *Xenobiotica* **26**:681–693.
- Palmer KJ and Spencer CM (1997) Zolmitriptan. *CNS Drugs* **7**:468–478.
- Perry CM and Markham A (1998) Sumatriptan—an updated review of its use in migraine. *Drugs* **55**:889–922.
- Renwick AB, Watts PS, Edwards RJ, Barton PT, Guyonnet I, Price RJ, Tredger JM, Pelkonen O, Boobis AR, and Lake BG (2000) Differential maintenance of cytochrome P450 enzymes in cultured precision-cut human liver slices. *Drug Metab Dispos* **28**:1202–1209.
- Rodrigues AD (1999) Integrated cytochrome P450 reaction phenotyping. Attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. *Biochem Pharmacol* **57**:465–480.
- Seiberras DG, Polvino WJ, Gertz BJ, Cheng H, Stepanavage M, Wittreich J, Olah T, Edwards M, and Mant T (1997) Initial human experiences with MK-462 (rizatriptan): a novel 5-HT_{1D} agonist. *Br J Clin Pharmacol* **43**:49–54.
- Shimada T, Yamazaki H, Mimura M, Inui Y, and Guengerich FP (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270**:414–423.
- Smith DA (2000) Induction and drug development. *Eur J Pharm Sci* **11**:185–189.
- Stewart M, Napier CM, Katugampola SD, McHarg AD, and Wallis RM (1999) The binding affinity and functional activity of eletriptan and other 5-HT_{1B/1D} agonists at the human recombinant 5-HT_{1B} and 5-HT_{1D} receptors, in *Proceedings of the British Pharmaceutical Society*, 8–9 April 1999, Poster Abstract P40.
- Tepper S, Allen C, Sanders D, Greene A, and Bocuzzi S (2003) Co-prescription of triptans with potentially interacting medications: a cohort study involving 240,268 patients. *Headache* **43**:44–48.
- Thummel KE and Wilkinson GR (1998) *In vitro* and *in vivo* drug interactions involving human CYP3A. *Annu Rev Pharmacol Toxicol* **38**: 389–430.
- Umberhauer DR, Lankas GR, Pippert TR, Wise LD, Cartwright ME, Hall SJ, and Beare M (1997) Identification of a P-glycoprotein-deficient subpopulation in the CF-1 mouse strain using a restriction fragment length polymorphism. *Toxicol Appl Pharmacol* **146**:88–94.
- Wandel C, Kim RB, Kajiji S, Guengerich FP, Wilkinson GR, and Wood AJJ (1999) P-Glycoprotein and cytochrome P-450 3A inhibition: dissociation of inhibitory potencies. *Cancer Res* **59**:3944–3948.