**Tariquidar, a Selective P-glycoprotein Inhibitor, Does Not Potentiate Loperamide’s Opioid Brain Effects in Humans despite Full Inhibition of Lymphocyte P-glycoprotein**

Daniel Kumin, M.D.,* Gabenga G. Sofowora, M.D.,* John P. Donahue, Ph.D.,† Usha B. Nair, Ph.D.,‡
Grant R. Wilkinson, Ph.D.,§ Alastair J. J. Wood, M.D.,§ Mordechai Muszkat, M.D.¶

**Background:** Loperamide, a potent opioid, has been used as an in vivo probe to assess P-glycoprotein activity at the blood–brain barrier, because P-glycoprotein inhibition allows loperamide to cross the blood–brain barrier and exert its central opioid effects. In humans, studies with nonselective and moderately potent inhibitors resulted in mild opioid effects but were confounded by the concurrent inhibition of loperamide’s metabolism. The authors studied the effect of the highly selective, potent P-glycoprotein inhibitor tariquidar on loperamide’s central opioid effects.

**Methods:** In a randomized, double-blind, crossover study, nine healthy subjects received on 2 study days oral loperamide (32 mg) followed by an intravenous infusion of either tariquidar (150 mg) or placebo. Central opioid effects (pupil diameter, sedation) were measured for 12 h, and blood samples were drawn up to 48 h after drug administration to determine plasma loperamide concentrations and ex vivo P-glycoprotein activity in T lymphocytes. Values for pupil diameter and loperamide concentrations were plotted over time, and the areas under the curves on the tariquidar and placebo study days were compared within each subject.

**Results:** Tariquidar did not significantly affect loperamide’s central effects (median reduction in pupil diameter area under the curve, 6.9% [interquartile range, –1.4 to 12.1%]; \( P = 0.11 \)) or plasma loperamide concentrations (\( P = 0.12 \)) but profoundly inhibited P-glycoprotein in lymphocytes by 93.7% (95% confidence interval, 92.0–95.3%).

**Conclusion:** These results suggest that despite full inhibition of lymphocyte P-glycoprotein, the selective P-glycoprotein inhibitor tariquidar does not potentiate loperamide’s opioid brain effects in humans.

THE penetration of drugs into the brain is often limited by the presence of a functional blood–brain barrier. One of its important components is the efflux transporter P-glycoprotein localized on the luminal side of the brain capillary endothelial cell. In certain clinical conditions, increasing cerebral drug concentrations without a concomitant change in systemic levels would be advantageous. One way of achieving this would be to impair brain P-glycoprotein by use of a pharmacologic inhibitor. Several proof-of-principle studies in rodents, using selective and potent third-generation inhibitors such as zosuquidar/LY-335979,1,2 elacridar/GF-120918,3–6 and tariquidar/XR-9576,7 have demonstrated increased central nervous system effects associated with enhanced drug concentrations in the brain—often many-fold. Recently, positron emission tomography imaging studies using C11-labeled probes in higher species (pigs and nonhuman primates)8,9 also demonstrated greatly increased brain levels of the labeled drugs after the administration of third-generation P-glycoprotein inhibitors, whereas a study in humans showed a more modest increase.10

Although positron emission tomography imaging is a powerful tool for such studies, it requires suitable probes and sophisticated and expensive instrumentation/facilities. An alternative and attractive approach that has been suggested is based on the use of a drug with intrinsic central activity which, however, under normal circumstances does not exhibit this characteristic because P-glycoprotein at the blood–brain barrier limits its entry into the brain. Accordingly, if the barrier’s function is reduced by inhibition of P-glycoprotein, the probe drug will presumably enter the brain and, under appropriate conditions, its central nervous system effects could be measured. Loperamide, a \( \mu \)-opioid receptor agonist with potency similar to that of morphine,11,12 has been used as such a probe in studies in mice lacking P-glycoprotein13–15 or in which the transporter’s function had been pharmacologically inhibited.7 In P-glycoprotein “knockout” mice [mdr1a (−/−)], loperamide brain concentration was increased 13- to 65-fold compared with wild-type mice, and loperamide induced severe central opiate toxicity (even at low doses) that was not observed in mice with intact P-glycoprotein.13,14 Administration of potent and selective third-generation P-glycoprotein inhibitors such as tariquidar and elacridar to mice with intact P-glycoprotein resulted in a dose-dependent increase in loperamide’s brain penetration and analgesic effects, transforming loperamide into a centrally active opiate.7

On the basis of the above assumptions and animal findings, the centrally mediated effects of loperamide have been used to investigate in vivo P-glycoprotein function at the blood–brain barrier in humans. Such studies have involved possible genetic regulation...
of P-glycoprotein activity,16,17 and drug interactions by potential or known P-glycoprotein inhibitors.18–20 However, these studies have been limited to P-glycoprotein inhibitors with low selectivity and potency (inhibition constant $K_i$ in the millimolar range), e.g., quinidine and ritonavir, that produce significant changes in loperamide’s metabolism and its systemic plasma concentrations,17–20 which confound interpretation of any changes in its central effects and, in addition, could produce significant adverse effects in vivo.

Potent and selective third-generation P-glycoprotein inhibitors such as tariquidar, which do not affect loperamide’s metabolism and have been demonstrated to produce pronounced and prolonged inhibition of P-glycoprotein function in various tissues,21–23 overcome the disadvantages of older inhibitors. Accordingly, we designed a study to examine the effects of tariquidar on loperamide’s disposition and central nervous system effects in humans. Our hypothesis was that at doses expected to produce extensive systemic P-glycoprotein inhibition, tariquidar would not affect loperamide’s disposition but would result in profound central opioid effects.

Materials and Methods

The studies were approved by the Vanderbilt University Institutional Review Board, Nashville, Tennessee, and each subject provided written informed consent. All subjects were healthy as determined by the absence of significant clinical abnormalities on medical history, physical examination, and routine laboratory tests and refrained from taking any medications for at least 1 week before the study.

Study 1: Dose-finding Study

Initially, an open-label dose-escalation study was performed to determine the dose of loperamide that could be safely coadministered with tariquidar and would produce measurable central opioid effects without clinically significant reductions in blood pressure or blood oxygen saturation. Fifteen white subjects (11 men), aged 26.9 ± 5.3 yr (mean ± SD) and with a body mass index of 24.9 ± 3.0 kg/m$^2$, were studied. After an overnight fast, subjects were first pretreated with tariquidar (Xenova Ltd., Slough, United Kingdom), 150 mg diluted in 500 ml dextrose, 5%, infused intravenously over 30 min. This regimen has been shown to completely block P-glycoprotein–mediated substrate efflux from lymphocytes for up to 24 h in healthy subjects.21 Thirty minutes after the completion of the tariquidar infusion, an oral capsule of loperamide (Spectrum Pharmacy Products, Tucson, AZ) prepared by the Vanderbilt University Medical School compounding pharmacy at a dose of 0.5, 1, 2, 4, 6, 8, 12, 14, 16, 32, or 48 mg was then serially administered on separate days to one subject (n = 3 at the 32-mg dose).

Central opioid effects were determined by changes in alertness and pupil size. A subjective visual analog scale (VAS) was used to evaluate level of sedation, with 0 representing “asleep” and 10 representing “wide awake.” In addition, short-term memory recall was assessed using a digit symbol substitution test (DSST).24 Pupil constriction was measured after 5 min of adaptation to dim light by an infrared pupillometer (RK-726PCI; ISCAN Inc., Burlington, MA). During the measurement, the subjects fixed their gaze at a dot placed at eye level 3 m in front of them. The pupillometer automatically collected 1,800 images of the eye over 30 s and determined the mean pupil size over this period. All three of the above measures were performed before and every 30 min for 12 h after loperamide administration. In addition, heart rate and blood oxygen saturation were monitored continuously by a bedside cardiac monitor (Dynamap, MPS; Johnson & Johnson Medical, Tampa, FL), which also was used to determine blood pressure every 15 min.

Study 2: Crossover Study

Study Design and Procedures. The second study was designed as a randomized, double-blind, two-way crossover study. On 2 study days separated by 14 days, subjects received an intravenous infusion over 30 min of either placebo (5% dextrose) or tariquidar (150 mg diluted in 500 ml dextrose, 5%). Thirty minutes after completion of the infusion, they swallowed a single oral dose of 32 mg loperamide (capsules prepared by the Vanderbilt University Hospital Investigational Drug Pharmacy). The nine subjects (eight men) consisted of seven white and two black subjects, aged 24.1 ± 4.4 yr (mean ± SD) and with a body mass index of 25.0 ± 4.1 kg/m$^2$. All studies were performed after an overnight fast, and subjects continued fasting until 4 h after the loperamide administration. Pupil diameter, sedation VAS, and DSST were evaluated, as described in the previous paragraph, before and every 30 min for 12 h after loperamide administration. In addition, serial blood samples (10 ml) were obtained through an indwelling catheter before and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 h after loperamide administration. One aliquot was collected in EDTA tubes; after separation, the plasma was stored at −20°C until analyzed for loperamide concentrations. Up to 24 h after loperamide administration, plasma from another aliquot was obtained from an acid–citrate–dextrose anticoagulated sample and stored at 4°C until used in the dye efflux procedure the following morning. Whole blood was also collected at baseline before the administration of tariquidar, maintained at room temperature, and used as a source of CD4$^+$ and CD8$^+$ T lymphocytes on the following morning.

Loperamide Concentrations. The plasma concentration of loperamide was determined by a validated...
positive ion, electrospray, tandem liquid chromatography–mass spectrometry as described by He et al. Briefly, this involved tert-butylmethyl ether extraction of a plasma sample buffered to pH 9.6 with sodium carbonate. The organic extract was then evaporated to dryness under nitrogen at 40°C, and the residue was reconstituted with 200 μl ammonium acetate:methanol (1:4), 20 ms. Gradient, liquid chromatography with 20 ms ammonium acetate–acetonitrile and a Luna C18 reverse-phase column (Phenomenex, Torrence, CA) was then used and the eluting compounds analyzed by a TSQ 700 mass spectrometer (Finnegan, San Jose, CA). Daughter ions m/z 477 → 226 and m/z 519 → 266 corresponding to loperamide and O-acetyl loperamide (internal standard) were monitored. The loperamide assay had an intraday relative SD of 2.1–4.6% and an interday relative SD of 8.3–14.5%.

Ex Vivo Lymphocyte P-glycoprotein Assay. The functional activity of P-glycoprotein in lymphocytes from each individual subject was determined by a dye efflux method. In brief, this involved loading cells with 3,3-diethyloxacarbocyanine iodide (DiOC₂(3)) by incubating 5 ml prestudy acid–citrate–dextrose anticoagulated whole blood with an equal volume of 100 nM DiOC₂(3) in Dulbecco phosphate-buffered saline (Invitrogen Corp., Carlsbad, CA) for 15 min at 37°C. Cells were separated by centrifugation at 400g, washed once with 10 ml ice-cold phosphate-buffered saline, and distributed in 40-μl aliquots to each well of a 96-well deep well microplate. Cells were subsequently incubated with 60 μl test plasma (the prestudy sample, the prestudy sample to which 50 μm verapamil had been added, or the individual serial plasma samples collected over 48 h) at 37°C for 60 min. Lymphocytes were then labeled with fluorescent-conjugated monoclonal antibodies (CD4-PE, CD8-PerCP-Cy5.5, and CD45RA-APC; BD Pharmingen, San Diego, CA) for 30 min on ice. Erythrocytes were lysed with Optilyse B according to the manufacturer's instructions (Immunotech, Marseille, France), and cells were pelleted by centrifugation at 400g for 5 min at 4°C. After washing with ice-cold phosphate-buffered saline, the cells were fixed with 2% paraformaldehyde in phosphate-buffered saline. The cellular DiOC₂(3) content was quantified by a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). Fluorescence was detected after excitation at 488 nm, through a 530-nm band-pass filter. Data were analyzed with WinMDI software, version 2.8 (J. Trotter, Scripps Institute, La Jolla, CA). Naïve cells were defined by positive surface staining for CD45RA. The percentage of total and naïve CD8 and CD4 T lymphocytes that effluxed DiOC₂(3) (% dim cells) was established by setting the gate between dim (dye efflux negative) and bright (dye efflux positive) cell populations based on the prestudy positive control sample with verapamil, which inhibited all DiOC₂(3) efflux in every subject (data not shown). For each test sample, the percentage inhibition was quantified by determining the percentage of dim cells and linearly interpolating this value in comparison with incubation with verapamil (100% inhibition) or without verapamil (0% inhibition). Repeat examination of successive lymphocyte batches from the same subjects (untreated) showed small assay variability (intraday and interday coefficients of variation, 1.6% and 2.8%).

Statistical Analysis

For every subject and study day, absolute pupil diameters (0–12 h), scores of the alertness test (0–12 h), and loperamide plasma concentrations (0–48 h) were plotted against time, and the areas under the curves (area under the loperamide concentration curve [AUCₕₜₜ, area under the pupil, VAS, and DSST effect curves [AUE_pupil, AUE_VAS, and AUE_DSST, respectively]) were calculated using the trapezoidal method (GraphPad Prism version 4; GraphPad Software, San Diego, CA) as the sum of the individual areas under each segment of the time response–concentration curve. For one subject, blood samples for the determination of loperamide concentrations and the lymphocyte P-glycoprotein assay were available only up to 12 h after dosing, and she was excluded from the statistical analysis of AUCₕₜₜ and lymphocyte P-glycoprotein activity. The paired Wilcoxon signed rank test was used for within-subject comparisons of the AUCs between the placebo and tariquidar study days (SPSS version 13; SPSS Inc., Chicago, IL). Data are presented as mean ± SD or median and interquartile range (IQR) for nonnormally distributed data. All tests were two-tailed, and P < 0.05 was considered statistically significant.

Results

Dose-finding Study

The purpose of this study was to determine the safety of loperamide when coadministered with the maximum single dose of tariquidar approved for human studies (150 mg). Over the oral loperamide dose range of 0.5–32 mg, no untoward effects were noted. However, in the single subject receiving 48 mg loperamide, systolic blood pressure gradually decreased by 30 mmHg 3 h post loperamide dose before returning to baseline within 8 h, and sedation, nausea, and constipation developed and gradually improved over 48–72 h. Accordingly, we studied two additional subjects with a dose of 32 mg, and no adverse effects were observed. Therefore, we chose a loperamide dose of 32 mg for the subsequent crossover study.

Crossover Study

No significant changes in blood pressure or other adverse events were noted in study 2, except for one...
subject who reported prolonged tiredness after receiving loperamide on both the tariquidar and placebo days.

**Pupil Constriction**

Baseline pupil diameters did not differ on the 2 study days \((P = 0.59)\). Tariquidar did not increase loperamide's miotic effects (fig. 1). There was no significant difference in pupil diameter \(\text{AUE}_{0-12}\) between the placebo and tariquidar days (median, 65.8 mm \(\times\) h [IQR, 44.7 to 80.9] and 49.4 mm \(\times\) h [IQR, 39.9 to 77.9], respectively; \(P = 0.11\); fig. 2), and the median percent decline in AUE was 6.9\% (IQR, –1.4 to 12.1). In fact, in six of the nine subjects, the pupil diameter–time curves on the tariquidar and placebo days were virtually superimposable (fig. 1). Only one subject (subject 4) showed pronounced pupil constriction after tariquidar, resulting in a decline in pupil diameter AUE of 36.5\%; this was accompanied by a marked increase in loperamide plasma concentrations after tariquidar, with \(C_{\text{max}}\) increasing from 3.7 to 8.9 ng/ml and the loperamide AUC increasing by 133\% (see Loperamide Pharmacokinetics section, last sentence).

**Alertness Scores**

No changes in alertness were observed between the placebo and tariquidar days of the study. For the VAS, the median \(\text{AUE}_{\text{VAS}}\) areas under the measure–time curves over 12 h were 34.6 cm \(\times\) h (IQR, 22.9 to 49.2) and 36.6 cm \(\times\) h (IQR, 20.4 to 49.2) on the placebo and tariquidar days, respectively \((P = 1.0)\), and there was no difference in minimal, maximal, median, or mean VAS score (all \(P > 0.58\)). Similarly, the median \(\text{AUE}_{\text{DSST}}\) (248 correct matches \(\times\) h [IQR, 232 to 271] and 244 correct matches \(\times\) h [IQR, 216 to 267]) on the placebo and tariquidar days, respectively; \(P = 0.48\) and minimal, maximal, mean, and median DSST scores were comparable between the 2 study days (all \(P > 0.48\)).

**Lymphocyte P-glycoprotein Inhibition Ex Vivo**

Dye efflux from lymphocytes obtained from subsequent blood samples during 24 h after placebo adminis-
tration was unaffected as compared with baseline (mean dye efflux at 24 h, 96.9 ± 8.4% of baseline; fig. 3). However, dye efflux from lymphocytes obtained after administration of tariquidar was dramatically reduced within 5 min of beginning the tariquidar infusion to a mean ± SD of 6.3 ± 2.0% of the baseline value, corresponding to a mean 93.7% (95% confidence interval, 92.0–95.3%; range, 90.0–96.7%) inhibition. During the 24 h after tariquidar administration, dye efflux from lymphocytes ranged between 6% and 13% of its baseline value, and remained low (9.0 ± 4.5%) at 24 h after tariquidar infusion (fig. 3), thus indicating prolonged and almost total inhibition of P-glycoprotein on lymphocytes.

### Loperamide Pharmacokinetics

The area under the loperamide plasma concentration–time curve (0–48 h) after tariquidar administration did not significantly differ from that after placebo (median AUC, 126.5 ng·h/ml [IQR, 100.3 to 156.6] and 87.8 ng·h/ml [IQR, 65.7 to 156.6] for tariquidar and placebo, respectively; \( P = 0.12 \)). However, in both phases of the study, there was considerable (11- to 16-fold) interindividual variability in the AUC of loperamide plasma concentrations. Visual inspection of the individual plasma concentration–time curve (fig. 4) suggested that in seven subjects coadministration of tariquidar did not substantially affect loperamide’s AUC, whereas in two subjects (4 and 8) it was markedly higher after tariquidar (increases of 133% and 104%, respectively). The maximum plasma concentration did not differ significantly on the 2 study days (median, 8.5 ng/ml [IQR, 7.3 to 10.3] and 5.7 ng/ml [IQR, 3.8 to 12.1] for tariquidar and placebo, respectively; \( P = 0.52 \)).

### Discussion

This is the first human study to use loperamide’s central nervous system effects (pupil constriction and sedation) as a measure of P-glycoprotein function at the blood–brain barrier after inhibition with a highly specific and potent third-generation P-glycoprotein inhibitor. Tariquidar did not significantly affect loperamide plasma concentrations, but fully inhibited P-glycoprotein activity in lymphocytes. In contrast, tariquidar did not affect loperamide’s central nervous system effects, suggesting, in keeping with our recent findings in mice, that P-glycoprotein localized at the blood–brain barrier is more resistant to inhibition than at the lymphocyte.7

### Differential P-glycoprotein Inhibition by Tariquidar

Previous studies in humans have demonstrated that after infusion of tariquidar at doses of 1.0–2.0 mg/kg body weight, comparable to the fixed dose (150 mg) administered in our study, P-glycoprotein mediated efflux of its substrate rhodamine-123 from lymphocytes was fully inhibited, and such inhibition was maintained for greater than 24 h.21 Moreover, uptake of the P-glycoprotein substrate \(^{99m}\)Tc-sestamibi into various tissues was also impaired after administration of a 150-mg dose of tariquidar,22 and studies in animals with comparable doses also suggested significant inhibition in many tissues. Therefore, the current observation indicating 94% mean inhibition of DiOC\(_2\) efflux from lymphocytes is entirely consistent with these previous findings. The surprising lack of effect of tariquidar on loperam-
The median difference between the placebo and tariquidar days in our main outcome, area under the pupil diameter curve (AUEpupil), was only 6.7% ($P = 0.11$), a difference much smaller in magnitude and statistical significance than that expected for a centrally active opiate with similar potency as morphine.

We have recently shown in animals that P-glycoprotein inhibition at the blood–brain barrier requires substantially greater doses/concentrations of inhibitor than other tissue sites, such as lymphocytes. Therefore, although the brain uptake of P-glycoprotein substrates such as nelfinavir, loperamide, and verapamil can be increased many-fold in animals by large dosages of inhibitor, these doses far exceed those attainable in the clinical setting. For example, tariquidar’s IC$_{50}$ for increasing loperamide’s brain:plasma concentration is 5.7 mg/kg in mice, approximately 19-fold higher than the IC$_{50}$ for P-glycoprotein inhibition in lymphocytes. Therefore, assuming that tariquidar potency is comparable in humans and rodents at the blood–brain barrier, as has been shown for cyclosporine, the standard tariquidar dose in humans (approximately 2 mg/kg), although sufficient to fully inhibit lymphocyte P-glycoprotein, is at the lower end of the dose–response curve for P-glycoprotein inhibition at the blood–brain barrier. The reasons for such organ-specific sensitivity to P-glycoprotein inhibition are unclear, but differences between lymphocytes and the blood–brain barrier in P-glycoprotein structure (e.g., “mini P-glycoprotein”), localization (e.g., caveolae), or expression may play a role.

**Loperamide as P-glycoprotein Probe**

Previous clinical studies used loperamide as an *in vivo* probe of brain P-glycoprotein function associated with different genetic variants of the transporter or after the coadministration of known P-glycoprotein inhibitors. These studies had several limitations, namely, the use of inhibitors (quinidine and ritonavir) that are not particularly potent or effective P-glycopro-

---

**Fig. 4.** Individual loperamide plasma concentration–time curves for the 48-h period after the administration of loperamide (32 mg) and either placebo (black dotted line) or tariquidar (gray solid line). Loperamide plasma concentrations were not available for subject 1 after the 12-h time point.
tein inhibitors but, in addition, impair loperamide’s metabolism and thus result in significantly elevated systemic drug levels.\textsuperscript{17–20} Further complicating data interpretation. To overcome these limitations, we used a selective and potent third-generation P-glycoprotein inhibitor, tariquidar, at a dose previously demonstrated to produce a marked decrease in transporter activity in various tissues, including brain and lymphocytes.\textsuperscript{21–23} In contrast to nonselective inhibitors, tariquidar does not inhibit CYP3A and thus does not affect loperamide metabolism. However, P-glycoprotein inhibition at the intestinal brush border could potentially increase loperamide’s bioavailability, as suggested for morphine.\textsuperscript{35} Although we did not find a statistically significant increase in loperamide AUC after tariquidar, with plasma concentration–time curves almost superimposable for seven of nine subjects, our study was not designed to examine this, and we cannot rule out small increases (<1.5-fold) in loperamide AUC in our study design.

To maximize the ability to detect a central opioid effect, we performed our study with the highest loperamide dose determined to be safe when coadministered with tariquidar (32 mg). This dose was 33–100% higher than the loperamide doses (16–24 mg) used in previous studies in which central effects of loperamide after the administration of a P-glycoprotein inhibitor have been reported.\textsuperscript{17,18} Nevertheless, an alternative explanation for the lack of central loperamide effects in the presence of full P-glycoprotein inhibition in lymphocytes in our study is that the dose and resulting plasma concentrations of loperamide were not sufficiently high to achieve effective brain levels, even if P-glycoprotein inhibition were sufficient to allow loperamide to penetrate into the brain.

Brain uptake of a P-glycoprotein probe depends both on its plasma concentrations and P-glycoprotein activity at the blood–brain barrier. Therefore, an ideal \textit{in vivo} probe should be safe to administer in dose producing plasma concentrations that result in a measurable central effect, and the plasma level–time profile should be relatively consistent between individuals. However, we found considerable interindividual variability in loperamide’s concentration–time profile. This is not entirely unexpected, because, besides being a substrate for P-glycoprotein, loperamide is extensively metabolized by CYP3A.\textsuperscript{34} As a result, the drug’s oral bioavailability would be expected to be low and highly variable.\textsuperscript{35}

**Conclusion**

The goal of achieving enhanced brain penetration of drugs in humans by inhibiting P-glycoprotein function at the blood’s brain barrier seems to be considerably more difficult than suggested by proof-of-principle studies in animals and human P-glycoprotein inhibition studies in other organs. A possible reason for this is greater resistance of brain P-glycoprotein to inhibition compared with other tissues, such as T lymphocytes, as we have previously shown in mice. Whether this holds true for other recently developed inhibitors, such as zosuquidar, elacridar, and laniquidar, which seem to have different mechanisms of interaction with P-glycoprotein,\textsuperscript{7} remains to be determined. The findings also suggest that, despite loperamide’s intrinsic characteristics as a putative centrally active opiate, other factors, including highly variable oral availability and a shallow dose–response curve, make it a less ideal probe for the \textit{in vivo} assessment of P-glycoprotein activity at the blood–brain barrier.

We are indebted to Gail Mayo, R.N. (Research Nurse, Division of Clinical Pharmacology, Vanderbilt University, Nashville, Tennessee), for her assistance with subject recruitment, blood sampling, and data collection.

**References**


23. Xenova: Tariquidar (XR-9576): P-glycoprotein Pump Inhibitor (investigator brochure). Slough, United Kingdom, Xenova, 2005