SUPPLEMENTAL MATERIALS AND METHODS

^{11}C-dLop Imaging

Dynamic 3D images were acquired on a High Resolution Research Tomograph (Siemens Medical Solution, Knoxville, TN) over 60 minutes. A six-minute transmission scan using a ^{137}Cs point source was performed prior to radiotracer injection for attenuation correction. ^{11}C-dLop was injected via a computer-controlled Harvard programmable pump (Harvard Apparatus, Holliston, MA). The injection rate was calculated using the DOSE program (NIH PET Department) after inputting the syringe size, desired dose of radioactivity, and injection duration (60 seconds).

For 21 of the 42 scans acquired, arterial blood was manually sampled at 15-second intervals for the first two minutes, then at 3, 5, 10, 20, 30, 45, and 60 minutes. Plasma ^{11}C-dLop was measured using radio-high-performance liquid chromatography (radio-HPLC). The plasma concentration of parent radiotracer was separated from its radiolabeled metabolites. Free fraction of ^{11}C-dLop in plasma was measured by ultrafiltration, as previously described (*1*).

Injected doses of radioactivity from ^{11}C-dLop administration were as follows: 735 ± 15 MBq (baseline), 722 ± 13 MBq (tariquidar, 4 mg/kg delayed), 707 ± 44 MBq (tariquidar, 6 mg/kg delayed), 676 ± 130 MBq (tariquidar, 2 mg/kg concurrent), 239 ± 2 MBq (tariquidar, 4 mg/kg concurrent), 736 ± 7 MBq (oral tariquidar), and 701 ± 60 MBq (disulfiram).

Tariquidar Preparation

Tariquidar was supplied by AzaTrius Pharmaceuticals (London, U.K.). Each mL of tariquidar solution contained 7.5 mg of tariquidar base as the dimesylate salt in a sterile solution of 20% ethanol / 80% propylene glycol, with a small amount of hydrochloric acid added.

Tariquidar solution was refrigerated (2 – 8 ºC), and protected from light, and allowed to warm to ambient temperature before dilution. The stock solution was filtered and then diluted in 5% dextrose solution at a concentration of 0.6 mg/mL for IV administration. The infusion bag and tubing were protected from light at all times.

The oral formulation of tariquidar was supplied as the freebase formulated in opaque white hard gel capsules (100 mg) containing 4% sodium lauryl sulphate and anhydrous lactose.

Oral Tariquidar Pharmacokinetic Study

Subjects were given tariquidar at doses of 500 mg PO (n = 3, 1 female, weight 84 ± 20 kg, age 31 ± 5.7 years), 1,000 mg PO (n = 3, 2 female, weight = 70 ± 13 kg, age = 26 ± 5.2 years), or 1,500 mg PO (n = 3, 2 female, weight = 70 ± 19 kg, age = 33 ± 4.9 years). Venous blood samples were taken prior to tariquidar administration and again at 30, 60, 90, 120, and 150 minutes and 3, 4, 5, 6, 7, 8, 9 10, 11, 24, 25, and 26 hours post-administration to measure total concentration of tariquidar in plasma. Subjects were monitored for changes in blood pressure, temperature, heart rate, respiration rate, and electrocardiogram tracing at regular intervals. Oral bioavailability of tariquidar was calculated as $(AUC_{oral} / AUC_{IV}) \bullet (Dose_{IV} / Dose_{Oral})$, using previously published pharmacokinetic data for IV tariquidar (*2*).

^{11}C-dLop Image Analysis

Pituitary ROIs were hand-drawn onto the PET images using subject MRI for reference. Pituitary uptake values were compared in the 4 subjects who underwent ^{11}C-dLop PET under both baseline and blocked (2 mg/kg IV concurrent administration) conditions.

LC-MS/MS Quantification of Tariquidar in Human Plasma

Plasma was drawn to measure total tariquidar concentration for 16 subjects (the three subjects who received 1500 mg oral tariquidar, the 12 subjects who received concurrent administration of IV tariquidar, and the one subject who received delayed administration of 4 mg/kg IV tariquidar). For the subjects who received concurrent IV tariquidar, plasma samples were drawn at three time points during the tariquidar infusion—immediately before radiotracer injection, immediately after radiotracer injection, and at the end of tariquidar infusion—and averaged to create a single concentration measurement per subject. The other four subjects had one plasma sample drawn at the time of ^{11}C-dLop injection. Free fraction of tariquidar was determined for 5 of the 10 subjects who received tariquidar at 2 mg/kg IV with concurrent administration of radiotracer.

N-(2-(4-(2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)phenylcarbamoyl)-4-ethoxy-5-methoxyphenyl)quinoline-3-carboxamide, an ethyl analog of tariquidar, was synthesized by ethylation of phenol precursor (*3*) and used as an internal standard (IS) for measuring tariquidar. Liquid chromatography/mass spectrometry (LC-MS/MS) (API 5000, AB Sciex, Foster City, CA) was tuned to measure the product ions *m/z* 335 and *m/z* 349 of tariquidar and IS, respectively. Samples were chromatographed at 50 °C on a C18 column (2 × 50 mm, 3 μm; Phenomenex, Torrance, CA) with a gradient of binary solvents (A: B; 400 μL/min), where A was 10 mM ammonium acetate and 0.1% acetic acid in water, and B was 0.1% acetic acid in acetonitrile. During the analysis, the pumps ran 80% A: 20% B for one minute and then a linear gradient reaching 35% A: 65% B over 2.5 minutes. After 1.25 minutes, the column was washed with 5% A: 95% B for 2 minutes and the mobile phase composition was then returned to the initial condition.

Stock solutions of tariquidar and IS were prepared in dimethyl formamide containing 1% acetic acid and stored at 4 °C. Plasma (100 μL) from a human subject that had been infused with tariquidar was mixed with a solution of IS (20 ng) in dimethyl formamide (50 μL) and water (200 μL) in Eppendorf tubes. The plasma was immediately (less than 15 min delay) mixed with IS solution and then stored (-75 °C) for later analysis. The calibration curve samples (62.5−2000 ng/mL) were similarly prepared by spiking tariquidar solution into control human plasma and then stored. For LC-MS/MS determination of total tariquidar concentration in plasma, the stored plasma samples were analyzed within two weeks of their preparation from respective study. However, there was one set of plasma samples which was analyzed outside this timeframe. Because of the added IS and the calibration curve samples that accompany each set of plasma samples, some delay in the analysis will not impact the quantification of tariquidar by LC-MS/MS. For free fraction determination, storage times ranged from 3 to 157 days.

On the day of the LC-MS/MS analysis, these samples were thawed and treated with 1 M *aq.* ammonium acetate (10 μL) and acetonitrile (600 μL) and then mixed by vortexing for five minutes. The precipitated protein was separated by centrifugation ($10^4 \times g$; 4 min) and a 200 μL-aliquot of the supernatant liquid was mixed with 800 μL of a solution of 1% acetic acid in 50% *aq.* acetonitrile. The samples were filtered (PTFE, 4 mm 0.2 μm; Supelco, Bellefonte, PA) and a known volume (2 μL) was injected onto LC-MS/MS. The LC-MS/MS peaks (*m/z* 335, 349) were detected at retention times 3.1 minutes and 3.2 minutes for tariquidar and IS, respectively. The calibration curve, a plot of plasma concentrations of tariquidar versus peak area ratios of tariquidar to IS, was linear ($r > 0.99$). The LC-MS/MS measurement of tariquidar in human plasma was reproducible, as shown from the multiple measurements of the same sample (e.g., 131.9 ± 0.8 ng/mL; RSD= 0.6%; $n = 4$). Four previously analyzed plasma samples (not spiked

with IS) were thawed and mixed with IS and tariquidar concentration was measured. The difference (%) in the tariquidar concentration between the two measurements was 1.3, 0.4, 1.6, and 4.3. Thus the between-day reproducibility of the method was satisfactory and, additionally, freezing and thawing did not alter the plasma tariquidar. The time interval for between-day reproducibility was 14 days for plasma samples from oral tariquidar and 11 days for samples from intravenous tariquidar.

Quantification of Free Fraction of Tariquidar

Using enough radioactivity (0.5 μCi/assayed sample) to keep the counting error below 5% at one standard deviation in samples with the least detected activity, the plasma free fraction (f_p) of [^{3}H]tariquidar was determined in 21 healthy participants, five of which had PET imaging with [^{11}C]dLop. Plasmas were collected, stored at-80°C, and all assayed on the same day along with control standard human pooled plasma. In brief, [^{3}H]TQ (1 mCi/mL, specific activity 80 Ci/mmol, American Radiolabeled Chemicals, St. Louis, MO) was diluted in phosphate-buffered saline (PBS; pH 7.4) to a specific concentration of 0.1 μCi/μL solution. Radiochemical purity was more than 99.2% as determined by instant thin layer chromatography (ITLC-SG- Glass microfiber, impregnated with silica gel. Varian. Lake Forest, CA, Cat No: SGI0001). At this concentration, 15.6 μL of [^{3}H]TQ (1.56 μCi) was mixed with 625 μL plasma and incubated for 10 minutes at room temperature. A 0.75 μg/μL solution of carrier TQ (20:80% ethanol: propyleneglycol, CAS # 206873-63-4, Xenova Ltd., UK) was diluted to 0.1 μg/μL in PBS; 50 μL of this solution was then placed in each Centrifree receiving cup (Centrifree, Merck Millipore, Billerica MA) prior to centrifugation to minimize adsorption of [^{3}H]TQ, in the filtrate, to its wall.

Samples were assayed during 8 centrifugation sessions for 20 minutes at 5000 g. Each session included at least three duplicate plasmas from three participants and one duplicate control standard plasma whose results were used to normalize the test samples from the various centrifugation sessions. Thus, replicates of 150 μL of the ultrafiltrates (and carrier) and 20 μL of the total plasma were sampled into liquid scintillation vials each containing 4 mL of Ultima Gold MV cocktail. After dispensing the samples into each vial, the pipette tip was rinsed with the sample in vial (a) and then in a second vial (b). All vials were mixed and radioactivity was measured on the liquid scintillation counter (LSC) for five minutes. The counts detected from the second (b) wash were added to the sample counts (a) to be included for calculations. Results from the LSC were corrected for count quenching. The counts per ultrafiltrate volume were corrected for the added carrier in the receiving cup, and then divided by the activity in the total plasma volume to determine the free fraction. The ratio of the average of all 8 standard control plasma f_p values from each centrifugation run to that of each individually observed standard sample f_p value was used as a correction factor. This correction factor was multiplied by the individual f_p value of each test sample to result in normalized f_p values with eliminated variability from multiple centrifugation runs (8 runs).

SUPPLEMENTAL RESULTS

Oral Tariquidar Pharmacokinetic Study

Oral tariquidar was tolerated at all doses given. The 1500 mg PO dose resulted in C_{max} of 190 ng/mL at 11 hours post-administration (Supplemental Fig. 2). Therefore, we selected this dose and time interval between administration and ^{11}C-dLop injection for the PET study. Area under the curve for the 500, 1000, and 1500 mg doses were 878, 2810, and 3870 ng • h • mL^{-1},

respectively. Using published AUC data from Wagner et al. (*2*) (mean AUC = 2870 ng • h • mL^{-1} from a mean dose of 148 mg), the bioavailability of oral tariquidar was calculated to be 12%.

REFERENCES

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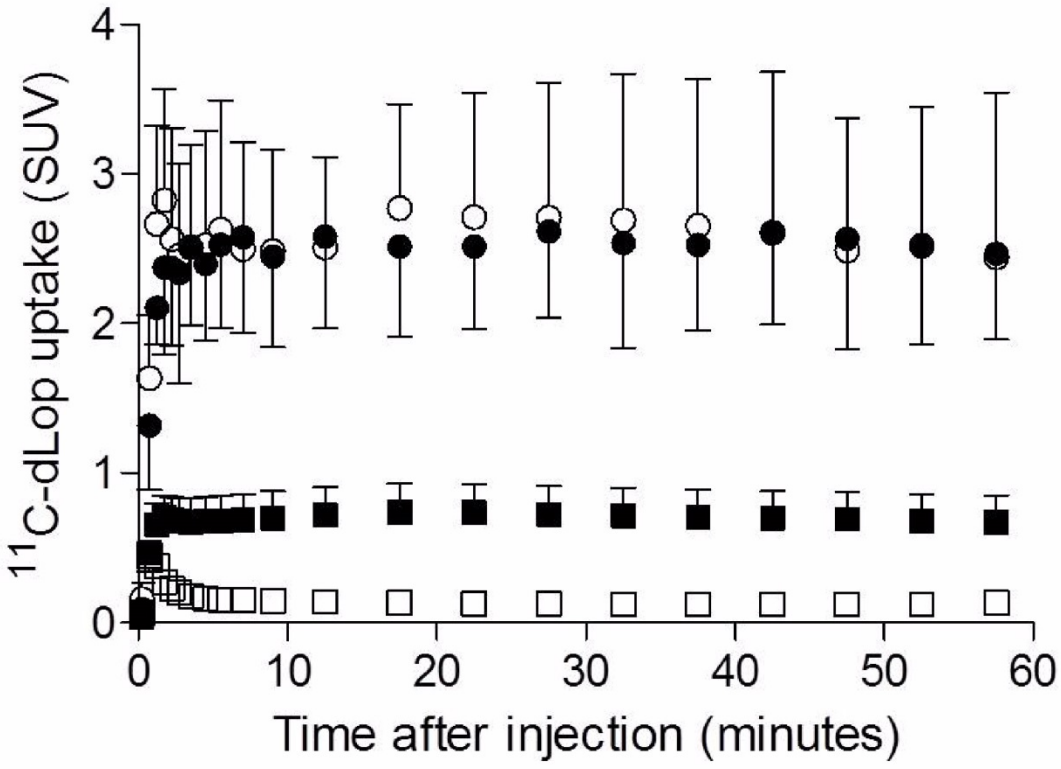
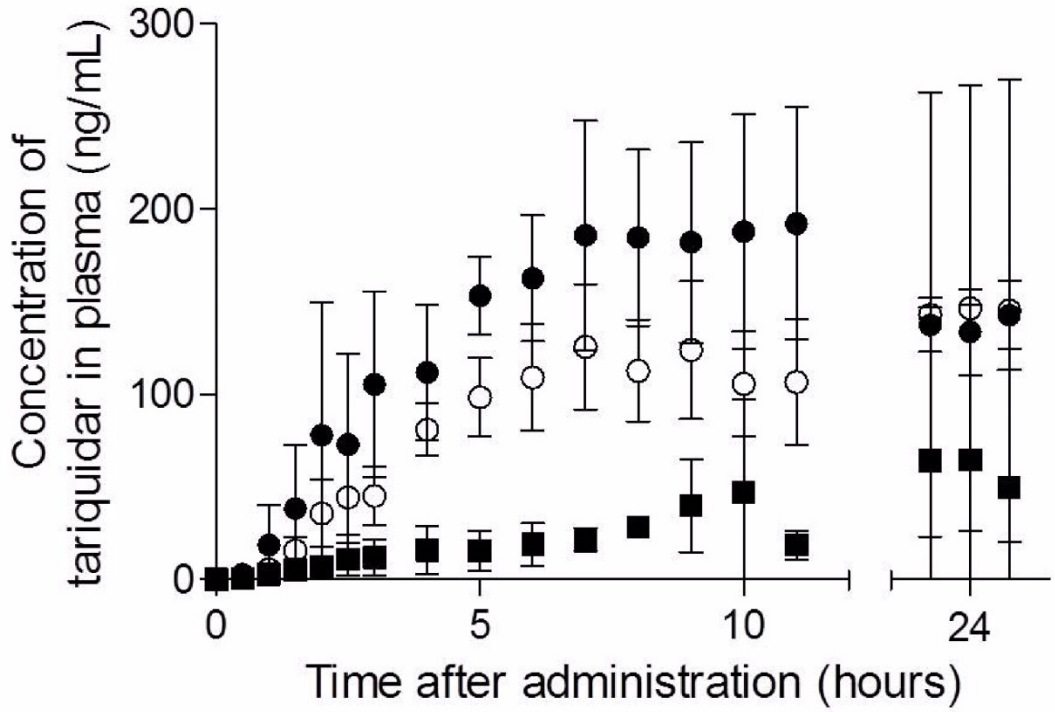


SUPPLEMENTAL FIGURE 1. ^{11}C-dLop uptake in pituitary gland at baseline (○), pituitary gland with concurrent administration of tariquidar 2 mg/kg IV (●), composite neocortex at baseline (□), and composite neocortex with concurrent administration of tariquidar 2 mg/kg IV (■) from 4 healthy subjects. Data given as mean ± SD.



SUPPLEMENTAL FIGURE 2. Total concentration of tariquidar in plasma after oral administration of tariquidar at 500 mg (●), 1,000 mg (○), and 1,500 mg (■). Data given as mean ± SD (n = 3 for each group).

Supplemental Table 1. Regional brain uptake of ^{11}C-dLop at baseline and after pharmacological inhibition of P-glycoprotein.

	Frontal Cortex	Parietal Cortex	Occipital Cortex	Temporal Cortex
Baseline (n=14)	2.88 ± 0.82	3.05 ± 0.65	3.15 ± 0.68	2.74 ± 0.59
Tariquidar				
2 mg/kg concurrent (n=10)	14.85 ± 5.17	16.12 ± 5.38	16.81 ± 5.91	14.34 ± 4.86
1500 mg oral (n=3)	1.90 ± 0.89	2.25 ± 1.06	2.73 ± 0.17	2.02 ± 0.94
6 mg/kg delayed (n=3)	9.76 ± 3.29	10.22 ± 3.21	11.34 ± 3.41	9.39 ± 3.12
Disulfiram				
2500 mg (n=3)	2.26 ± 0.13	2.73 ± 0.17	2.59 ± 0.23	2.37 ± 0.18

Data given as mean ± SD for area under brain time-activity curve from 10 to 30 minutes.