Supplemental Data

SUPPLEMENTAL METHODS

PET Imaging

Animals were fasted overnight, anesthetized for positioning in the PET scanner and kept under isoflurane (0.5%-2.0%, v/v) anesthesia during the entire study. The femoral artery and both femoral veins of the macaques were cannulated for blood sampling, radioactive tracer administration, and CsA infusion, respectively. Whole body images were acquired as 2D dynamic emission scans on an Advance Tomograph (GE Healthcare, Waukesha, WI). A 15 min transmission scan by use of a rotating germanium-68 source was followed by a sequence of 5 substudies, as described below.

[^{15}O]-water (45-76 MBq/kg) was administered as an intravenous bolus to measure tissue blood flow. Images were acquired and arterial blood samples (0.5 mL) were obtained as detailed in previous reports (*9, 10*). Blood and plasma radioactivity from this study and subsequent substudies, was measured by a gamma counter (Cobra Counter; Packard Corporation, Meriden, Conn).

Approximately 15 min after the [^{15}O]-water administration, [^{11}C]-verapamil (14-75 MBq/kg) was administered intravenously over 1 min. Image acquisition was conducted for up to 45 min after injection and frequent blood samples (0.5 mL) were taken to match the imaging sequence. Plasma [^{11}C]-verapamil and metabolite concentrations were determined as previously described (*13*).

CsA infusion (6, 12 or 24 mg/kg/h, UWMC Drug Services) was initiated after the completion of the first [^{11}C]-verapamil study and continued during the second [^{11}C]-verapamil and [^{15}O]-water study, for a maximum of 2h. The [^{15}O]-water and [^{11}C]-verapamil imaging sub-studies were repeated at ~45 min and ~1 h, respectively, after beginning the CsA infusion. Frequent blood samples were taken following the start of CsA infusion to measure CsA concentrations by

liquid chromatography–mass spectrometry (*10*).

At the end of the second [^{11}C]-verapamil study, [^{11}C]-CO (7.4-92.7 MBq/kg) was administered by inhalation to determine tissue blood volume. Images were acquired for 12 min after inhalation, and radioactivity was measured in blood samples (0.5 mL) taken at 6, 8 and 10 min. Each animal had an MRI scan within 2 weeks of the PET study to provide anatomical information for constructing regions-of-interest (ROI).

Image Reconstruction and Analysis

PET images were reconstructed using a 3D reconstruction algorithm with correction for scattered and random coincidences. The tomograph, dose calibrator, and gamma counter were cross-calibrated to express all measurements in common units of radioactivity (μCi/ml or Bq/ml). MRI images (T1 and T2) were co-registered to the PET images by use of conventional image-processing software (Alice [HIPG]; PMOD Version 3.0 (PMOD Technologies, Zurich, Switzerland). Regions of interest (ROIs) were identified on the co-registered MR images and summed [^{11}C]-verapamil images. Regions smaller than three times the machine resolution (1.2 cm) were corrected for partial volume. The ROIs from contiguous slices were combined to create volumes of interest (VOI) for each tissue type. VOIs were applied to both the dynamic image sets and the static summed SUV images for data extraction. Image and plasma data were decay-corrected to the injection time.

SUPPLEMENTAL RESULTS

Distribution of [^{11}C]-verapamil into maternal brain

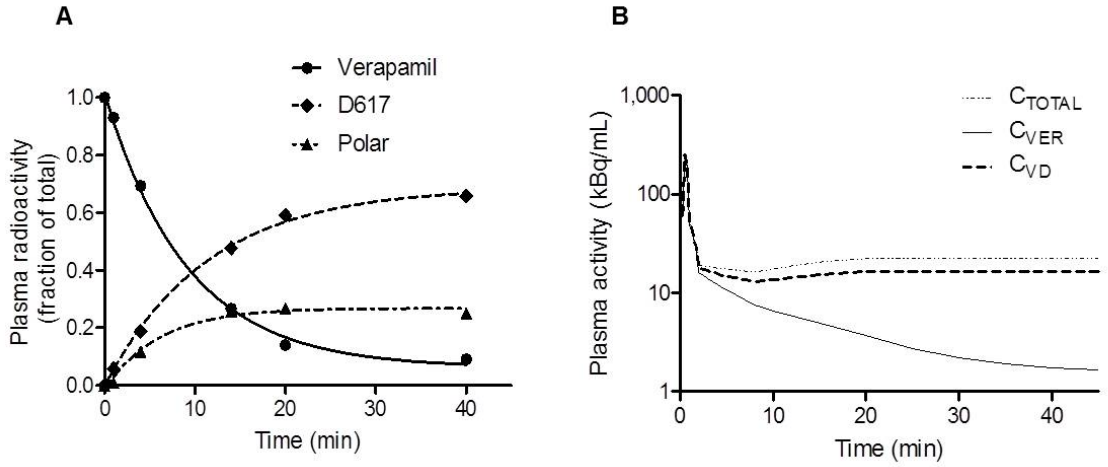
Over the 45 min of the study duration, metabolism of [^{11}C]-verapamil generated significant amount of circulating radio-labeled metabolites, namely D617 (or a metabolite that co-eluted with D617) and unknown polar metabolites (Supplemental Fig. 1A shows the profiles for macaque 7). To determine the influence of tracer metabolism on the estimation of kinetic parameters, several arterial input functions including or excluding metabolites were evaluated (see Methods). In contrast to the commonly accepted notion that the major metabolite D617 is also a P-gp substrate and has the potential to be transported between plasma and tissue, when the input function of combined verapamil and D617 (C_{VD}) was used, the fit of $2C_{45}$ model to during-CsA brain activity curve was poor (Supplemental Fig. 2, $2C_{VD\text{-}45}$). The AIC values of the $2C_{45}$ model when fitted to both pre-CsA and during-CsA brain time-activity curves were larger when C_{VD} vs. C_{VER} was used as the input function (pre-CsA: $p=0.048$; during-CsA: $p=0.003$, C_{VER} vs. C_{VD}, $n=12$). Then, we also evaluated the $2C_{45}$ model with total plasma activity as the input function, which took into consideration contributions from all metabolites. However, the model fit with this input function was the worst (Supplemental Fig. 2, $2C_{TOTAL\text{-}45}$).

Distribution of [^{11}C]-verapamil into fetal liver

In contrast to maternal brain, a shorter duration of the tissue scans up to 9 min for fetal liver was not considered because it failed to estimate the efflux rate constant k_2 with acceptable precision (COV% > 200%). Nonetheless, distributional clearance K_1 was well-estimated with COV% of less than 15%. Moreover, neither K_1 values (pre and during-CsA) nor the percentage change in K_1 differed significantly when different durations (9 min vs. 20 min vs. 45 min) of tissue activity data were used ($p>0.12$ in all comparisons).

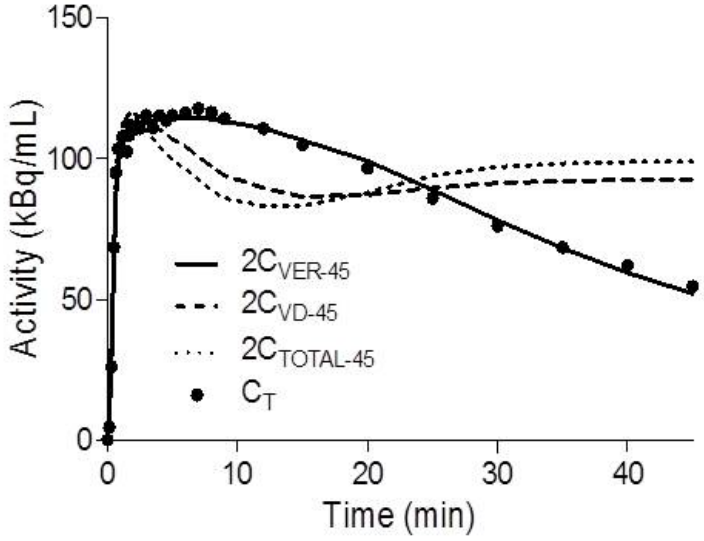
When 1C model was fitted to up to 20 min and 45 min of during-CsA dynamic data, systemic deviations of the model fit from the data occurred in several macaques. A more complex 2C model did not reduce the AIC values further or significantly (AIC-$1C_{20}$: 206.6 ± 45.2 vs. AIC-$2C_{20}$: 209.5 ± 42.8, n=11, data not shown). The lack of good model fit suggested that an intermediate placental compartment between plasma compartment and fetal liver compartment may be needed. However, the parameters of such a model would not be identifiable and was not pursued further.

SUPPLEMENTAL FIGURE 1.



SUPPLEMENTAL FIGURE 1. A) [^{11}C]-verapamil and its metabolite content in the plasma. To derive the fraction of verapamil in plasma as a function of time, an exponential function was fitted to the 40 min verapamil data. B) The verapamil input function, C_{VER}, was obtained from the product of the total plasma radioactivity, C_{TOTAL}, and the fraction of verapamil determined from plasma metabolite analysis. The primary circulating radio-labeled metabolite D617 is a P-gp substrate and is thought to behave like verapamil, and was therefore combined with the verapamil fraction to generate an alternative plasma input function (C_{VD}).

SUPPLEMENTAL FIGURE 2.



SUPPLEMENTAL FIGURE 2. A two-tissue compartment (2C) model was fitted to during-CsA brain time-activity curve (C_T) up to 45 min. Unchanged verapamil (C_{VER}), verapamil and D617 combined (C_{VD}) or total [^{11}C] activity (C_{TOTAL}) were used as the arterial input functions.