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Simultaneous PET Imaging of P-Glycoprotein Inhibition in Multiple Tissues in the Pregnant Non-Human Primate

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Abstract

Studies in rodents indicate that disruption of P-glycoprotein (P-gp) function increases drug distribution into tissues such as the brain and the developing fetus. To simultaneously and serially evaluate the impact of P-gp activity and inhibition on the tissue distribution of drugs in a more representative animal model, we tested the feasibility of conducting whole-body PET imaging of the pregnant non-human primate (*Macaca Nemestrina*). We used [¹¹C]-verapamil as the prototypic P-gp substrate and cyclosporine A (CsA) as the prototypic inhibitor.

Methods—Four pregnant macaques (gestational age 145–159d, term is 172d) were imaged after IV administration of [¹¹C]-verapamil (30–72 MBq/kg) before and during intravenous infusion of CsA (12 or 24mg/kg/h, n=2 each). The content of verapamil and its metabolites in plasma samples was determined using a rapid solid-phase extraction method. Plasma and tissue time-[¹¹C]-radioactivity concentration curves were integrated over 0–9 minutes after each verapamil injection. The area under the time-concentration curve (AUC)_{tissue}/AUC_{plasma}, served as a measure of tissue distribution of [¹¹C]-radioactivity. CsA effect on [¹¹C]-radioactivity distribution was interpreted as P-gp inhibition. The change in fetal liver AUC ratio served as a reporter of placental P-gp inhibition.

Results—CsA effect on tissue distribution of [¹¹C]-radioactivity (AUC ratios) did not increase with its mean blood concentration, indicating a near-maximal P-gp inhibition. CsA increased maternal brain and fetal liver distribution of [¹¹C]-radioactivity by 276%±88% (*P*<0.05), and 122%±75% (*P*<0.05) respectively. Changes in other measured tissues were not statistically significant.

Conclusion—These data demonstrate, for the first time, the feasibility of simultaneous, serial, non-invasive imaging of P-gp activity and inhibition in multiple maternal organs and the placenta in the non-human primate. Our findings, consistent with previous data in rodents, indicate that the activity of P-gp in the placenta and the blood-brain barrier is high and that its inhibition facilitates drug distribution across these barriers.

Keywords

P-glycoprotein; [¹¹C]-verapamil; blood-brain barrier; cyclosporine A; pregnancy

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Introduction

P-glycoprotein (P-gp) is a major efflux transporter that affects drug pharmacokinetics and pharmacodynamics. The importance of P-gp results from its broad substrate selectivity and its strategic localization at critical sites for drug pharmacokinetics, including the blood-brain barrier (BBB), placenta, intestine, liver, and kidneys (1–3). Studies in rodents with genetically or chemically disrupted P-gp function provide evidence for the role of P-gp in the pharmacokinetics of many drugs, including chemotherapeutic, antiretroviral, and cardiac agents (1,3). In each case, the greatest impact of P-gp on drug distribution is at the BBB and the placenta. For example, chemical inhibition of P-gp function in pregnant mice by the CsA analogue PSC833, increases the accumulation of digoxin in the maternal brain 8-fold, in the fetus up to 2.8-fold, but the magnitude of change in the maternal liver, kidneys and spleen is much less (4).

It has been widely assumed that P-gp in the human BBB and the placental barrier is as important as in rodents. In addition, it is commonly accepted that inhibition of P-gp may result in significant increase in tissue distribution of P-gp substrate drugs thereby increasing the efficacy or toxicity of drugs such as the anti-HIV protease inhibitors and cancer chemotherapeutic agents. However, since species can differ in the level of expression and activity of transporters, it is currently unknown whether this assumption is correct. We have begun to investigate this issue using positron emission tomography (PET) as a non-invasive method to assess P-gp function at the human BBB. Using [^{11}C]-verapamil as the P-gp substrate, and CsA as the P-gp inhibitor, we found that the brain distribution of [^{11}C]-radioactivity increases by 88% with average CsA blood concentrations of 2.8 μM (5). This is less than that predicted by genetic ablation or CsA inhibition of P-gp in rodents (2,6,7). When rats are studied at the same blood concentration of CsA as that achieved in our human study, the inhibition of P-gp-mediated transport at the BBB is similar (7). However, the maximal magnitude of P-gp inhibition by CsA at the human BBB cannot be assessed due to concerns of CsA toxicity.

Placental P-gp can limit drug delivery to the fetus such as in the case of the anti-HIV protease inhibitors. Alternatively, the barrier may protect the fetus against the effect of maternal cancer chemotherapy, such as doxorubicin, which has been safely used in pregnancy (8). Thus more detailed knowledge of P-gp function at the blood-placental barrier is important in drug therapy of pregnant women. However, due to differences in placental physiology, rodents may not be a good model for drug transfer across the human placenta (9). Moreover, non-invasive measurement by PET of placental P-gp function in humans is not possible due to fetal radiation exposure. Although we and others have previously shown that the level of P-gp expression in the human placenta decreases with gestational age (10,11), assessment of its function at various gestational ages is not possible in humans. When compared with rodent models, the pregnant non-human primate provides a more representative model for the study of maternal-fetal transfer of drugs (12–14). Therefore, using [^{11}C]-verapamil as the model P-gp substrate and CsA as the inhibitor, we tested the feasibility of non-invasive, simultaneous PET imaging of P-gp activity and its inhibition in maternal organs and in the placenta in the pregnant non-human primate, *Macaca nemestrina*. Our hypothesis was that P-gp inhibition by CsA would result in significant increase in distribution of [^{11}C]-radioactivity into tissues most protected by P-gp, i.e. the maternal brain and the fetus.

Methods

Chemicals and reagents

Racemic verapamil was purchased from Sigma-Aldrich (St Louis, Mo). D-617 (3,4-dimethoxy- α -[3-(methylamino)propyl]- α -(1-methylethyl)-benzeneacetonitrile) and norverapamil were kindly supplied by Knoll AG (Ludwigshafen, Germany). Palladium on

alumina (0.5%), anhydrous acetonitrile, and zinc (99%) were purchased from Aldrich Chemical Co (Milwaukee, WI). Ethanol, anhydrous sodium sulfate, and phosphate-buffered saline solution (PBS) were United States Pharmacopeia products. All other chemicals were purchased from multiple vendors and were of the highest chemical purity available.

Radiopharmaceuticals

[¹⁵O]-water, [¹¹C]-CO and racemic [¹¹C]-verapamil were synthesized as previously reported (15). The radiochemical purity of the radiopharmaceuticals was >95% with the specific activity of [¹¹C]-verapamil of, greater than 57 GBq/μmol.

Animal preparation

All experimental procedures were approved by the University of Washington Animal Care Committee. Four pregnant macaques were included in the study (Table 1). After an overnight fast, the animal was sedated with an intramuscular injection of ketamine (10 mg/kg) and atropine (0.04 mg/kg). After the animal was placed on a custom-built platform to ensure immobilization in the PET scanner (and also later during the MRI study), the animal was administered IV propofol (5 mg/kg) and intubated. Then, under isoflurane (0.5–2.0%; v/v) anesthesia, the femoral artery and both femoral veins were cannulated for blood sampling, radioactive tracer administration and CsA infusion, respectively. To reduce spillover of radioactivity from urinary excretion of the tracers, the animal was equipped with a urinary catheter and well hydrated. Hydration was provided by intravenous infusion of Normosol-R (a non pyrogenic, isotonic electrolyte solution). Prior to positioning the animal in the scanner, a bedside ultrasound was conducted to determine fetal position and heart rate. During the entire study, the animal was monitored non-invasively for blood oxygen saturation, body temperature and blood pressure.

PET imaging protocol

All PET studies were performed in a 2-dimensional acquisition mode on a General Electric PET Tomograph (Advance; GE Medical Systems, Waukesha, WI) providing 35 image planes over a 15-cm axial field of view (16). To include both the maternal brain and the fetal compartment in the field of view, the animal was positioned on its back, transverse to the scanner axis. This allowed dynamic imaging of the maternal brain, fetus, and all major maternal organs. At the beginning of each study, a 15-minute attenuation scan was acquired with a rotating germanium 68 source. The attenuation scan was repeated if there was any significant change in the position of the animal noted between the substudies. Each substudy is described in detail in Fig. 1 and briefly outlined below. The animals were administered 47–73 MBq/kg of [¹⁵O]-water (5 mL; IV, to measure tissue blood flow) as saline and immediately afterwards image acquisition was initiated, and arterial blood samples (0.5 mL) were obtained (Fig. 1). Blood radioactivity was counted utilizing a gamma counter (Packard Corp., Meriden, CT). At 5 to 15 minutes after the [¹⁵O]-water study, [¹¹C]-verapamil (34–72 MBq/kg), was infused intravenously over a period of 1 minute in a total volume of 5 to 10 mL of isotonic saline containing <13% (v/v) (less than 1.3 mL) ethanol. At the onset of tracer infusion, a 45-minutes dynamic sequence was started and blood samples (0.5 mL) were taken (Fig. 1). From these samples, aliquots of plasma (100 μL) were counted. A larger volume of blood (3–5 mL) was collected at 1, 4, 8, 14, 20 and 40 minutes to determine plasma verapamil and metabolite concentrations by solid-phase extraction (SPE). At the completion of [¹¹C]-verapamil scanning, CsA was administered as an infusion (12 or 24 mg/kg/h) for a maximum of 2 hours. A second [¹⁵O]-water imaging study (injected dose 48–76 MBq/kg) was conducted at approximately 45 minutes after initiating CsA infusion, to measure any changes in tissue blood flow induced by CsA. After 1 hour of CsA infusion, [¹¹C]-verapamil administration was repeated (30–66 MBq/kg) followed by an identical imaging and blood sampling sequence.

described above. To measure blood CsA concentrations, blood samples (1 mL) were taken at 1,4,8,14,20, and 40 minutes after the start of second [^{11}C]-verapamil infusion. CsA blood concentrations were determined by LC-MS by the Department of Laboratory Medicine, University of Washington. At the end of the second [^{11}C]-verapamil substudy, [^{11}C]-CO, was administered by inhalation to determine tissue blood volumes (Fig. 1).

The above protocol was used in three animals and was modified in the fourth animal to reduce compression of central vessels by the pregnant uterus as follows: The animal was positioned in lateral decubitus position; the duration of the second [^{11}C]-verapamil substudy was shortened to 25 minutes; [^{11}C]-verapamil administration was initiated 30 minutes into CsA infusion (12 mg/kg/hr CsA); and the [^{11}C]-CO study was not performed.

Reconstruction of PET images

After correction for random coincidences, scatter and attenuation, images were reconstructed onto a 128×128 matrix of 35 slice volumes using a 2D filtered back projection method with a 12mm Hanning filter. Standardized uptake value (SUV) image sets were created by summing the dynamic data from 0 to 5 minutes for [^{15}O]-water, 1 to 9 minutes for [^{11}C]-verapamil and 4 to 12 minutes for [^{11}C]-CO to facilitate region-of-interest (ROI) placement for image analysis. The tomograph, dose calibrator, and gamma counter were cross-calibrated to express all measurements in common units of radioactivity (μCi or MBq).

Image analysis

Within 2 weeks of the PET study, the animal was imaged (in the same position as in the PET scanner) with a 1.5-T MR instrument (Singa; GE Healthcare). Regions of interest (ROIs) were identified on MRI T1- and T2-weighted images and summed [^{11}C]-verapamil images. Using the MRI images and PET transmission scans as guides, the partial volume-corrected (17) ROIs from contiguous slices were combined to create volumes of interest (VOIs) for each tissue type using conventional image processing softwares (Alice image processing software, HIPG, Boulder, CO or PMOD, version 2.9; PMOD Technologies, Zurich, Switzerland). VOIs were applied to both the dynamic image sets and the static summed SUV images for data extraction.

To better facilitate visualization of the effect of CsA, images of the first study (without CsA) scans were subtracted from those of the second study (with CsA) scans (PMOD), after normalization to injected dose and co-registration to compensate for the effect of any animal motion between scans. This algorithm allowed visualization on a pixel-by-pixel basis of the net effect of CsA on tissue distribution of [^{11}C]-radioactivity.

Estimation of tissue blood flow

Image-based arterial input functions from dynamic [^{15}O]-water studies were created from cardiac ROIs over the maternal heart derived by segmentation (18) and scaled by radioactive measurements of late arterial blood samples. A one compartment model (19) using the input function and the dynamic image sequence were used to generate parametric image volumes of blood flow (20). Manual placement of specific tissue ROIs were used to recover the average blood flow for each region.

Quantification of verapamil and its metabolites in plasma

The amount of radioactive verapamil and its metabolites in the plasma was determined after solid-phase extraction (SPE) and HPLC, as previously described (5,21).

Data and statistical Analysis

For tissue or arterial plasma area under the curve (AUC) analysis, image and plasma data were decay-corrected to the injection time before curve integration. Then the ratio $AUC_{\text{tissue}} : AUC_{\text{plasma}}$ was computed. This AUC ratio served as a measure of tissue distribution of [^{11}C]-radioactivity. The effect of CsA on this distribution was interpreted as P-gp inhibition. Data were expressed as mean $\pm$ SD. The difference between studies performed in the absence or the presence of CsA was analyzed by the Mann-Whitney test. Significance was set at $P < 0.05$.

Results

The first two animals tolerated the imaging protocol with no adverse events and delivered full term viable babies. In these animals, [^{11}C]-verapamil injection and CsA infusion did not affect blood pressure or heart rate. One animal that was positioned on her back and treated with 24 mg/kg/hr CsA experienced significant blood pressure fluctuations at the end of the study after imaging had been completed. Eight days following the PET study, this animal underwent a C-section to deliver a fetus that was diagnosed as nonviable by ultrasound. Following this adverse event, and suspecting that the fetal abortion may have been associated with the late blood pressure changes during the imaging study, the fourth animal was positioned on her left side to avoid central vessel compression, and the imaging protocol was modified as described in the Methods section. The fourth study was completed successfully, without any adverse events in the mother or her fetus.

The blood concentrations of CsA (Fig. 2) were relatively constant at 12 mg/kg/hr but not at 24 mg/kg/hr rate. Although the number of animals was too small to statistically compare the effect of CsA dose on percent of plasma radioactivity consisting of verapamil or its metabolite, these percentages appeared to be unaffected by CsA dose (Supplementary data, Table 1). For this reason, we averaged the results across all 4 animals (Fig. 3). Due to the shortened protocol of the fourth study, results at 40 minutes represent 3 animals and have not been analyzed statistically.

Our rapid SPE method did not separate D-617 (3,4-dimethoxy- α -[3-(methylamino)propyl]- α -(1-methylethyl)-benzeneacetonitrile) from its O-demethylated metabolite, D-717. As in humans, only the dealkylated metabolites, D-617/D-717, and unknown polar metabolite(s) were observed in the plasma (Fig. 3). At 40 minutes following each injection, irrespective of CsA presence or its dose, verapamil accounted for less than 20% of plasma radioactivity. However, at 9 minutes, verapamil accounted for $48\% \pm 12\%$ and $44\% \pm 13\%$ of total plasma radioactivity in the absence and presence of CsA, respectively ($n=4$, Fig. 3). At the same time, D-617/D-717 accounted for $21\% \pm 7\%$ and $36\% \pm 11\%$ of total plasma radioactivity, respectively ($n=4$). To minimize the influence of metabolism in the analysis of tissue verapamil radioactivity, we chose to integrate the plasma and the tissue concentration-time curves over the first 9 minutes (AUC_{0-9}). At 5 minutes, the mid-interval of this period, mean blood CsA concentrations were 4.7 and 8.2 μM for the 12 mg/kg/hr CsA and 17.9 and 21.0 μM for 24 mg/kg/hr CsA infusion rates (Table 2).

For both CsA infusion rates, the profiles of injected dose (ID)-normalized plasma [^{11}C]-time-activity curves did not differ significantly in the absence and presence of CsA up to 40 minutes from verapamil injection (Fig. 4). In each animal, CsA administration increased the distribution of [^{11}C]-verapamil into maternal brain and fetal liver (reporter of placental P-gp activity) (Figs 4). In the maternal brain, CsA increased the ID-normalized peak concentration (C_{max}), time to peak concentration (t_{max}) and the ID-normalized AUC_{0-9} by $209\% \pm 42\%$ ($n=4$, $P < 0.05$), $833\% \pm 410\%$ ($P < 0.05$) and $238\% \pm 49\%$ ($P < 0.05$), respectively; Supplementary data, Table 2). The fetal liver ID-normalized AUC_{0-9} increased by 108% ($n=4$, $P < 0.05$) following CsA administration (Supplementary data, Table 2). CsA did not significantly affect ID-normalized

AUC₀₋₉ of other tissues including the maternal liver and gallbladder. The impact of CsA on the uptake of radioactivity into maternal brain and fetal liver is shown in Fig. 5.

A better measure of the effect of CsA on uptake of [¹¹C]-radioactivity across blood-tissue barriers is obtained when the AUC values of individual organs are normalized by plasma AUC values (Fig. 6). CsA effect on AUC ratios did not increase proportionally with its mean blood concentrations (Table 2). As a result of P-gp inhibition at the macaque BBB, the maternal brain distribution of [¹¹C]-radioactivity increased from 0.92±0.15 to 3.40±0.66 (276%±88%; n=4, *P*<0.05). The AUC ratios of the fetal liver across all animals increased from 0.90±0.25 to 1.87±0.34 (122%±75%; *P*<0.05, n=4). AUC ratios of other tissues were not significantly affected by CsA. Comparable results were obtained when tissue and plasma concentration-time curves were integrated over the first 0–20 minutes or 0–40 minutes from each verapamil administration (Fig. 6). Changes at 40 minutes were not statistically tested because only 3 animals continued the imaging study beyond 20 minutes.

CsA increased mean maternal cerebral blood flow by 18%±38% (from 1.57±0.57 to 1.65±1.16 mL/cc/min, n=3). On average, CsA decreased placental blood flow by 19%, but inter-subject variability was high (SD=56%).

Discussion

Here, we report the first simultaneous assessment of *in vivo* P-gp activity and inhibition in multiple tissues in the non-human primate. Our study was designed to test the feasibility of whole-body imaging to identify tissues where inhibition of P-gp activity (by CsA) results in marked changes in tissue distribution of a P-gp substrate drug, [¹¹C]-verapamil. As per our studies and those of others, the percent change induced by CsA in the tissue distribution of [¹¹C]-verapamil radioactivity was interpreted as the magnitude of P-gp inhibition (2,5–7).

Because this is a pilot study that compares P-gp inhibition among different organs, we targeted two different blood concentrations of CsA, one that approximates the EC₅₀ for P-gp inhibition in the rat (7.2 μM) (7) and one that is higher than the rat EC₅₀ and predicted to completely inhibit P-gp function (7). Although verapamil undergoes stereoselective metabolism (22,23) its R and S enantiomers have approximately equal affinity for P-gp (24). Thus, P-gp-mediated distribution of [¹¹C]-verapamil radioactivity into tissues would be dependent only on the total [¹¹C]-racemic verapamil blood concentration and the tissue-specific P-gp activity, making it unnecessary to study the distribution of individual enantiomers.

The metabolism of verapamil in the pregnant non-human primate was faster than in humans (Fig. 3) (5) or in male macaques (25) and most likely enhanced by pregnancy, as a result of CYP3A induction (26,27). Similar to humans, the major radioactive metabolites were [¹¹C]-D617/D-717, formed by dealkylation, and polar metabolites formed by N-demethylation. CsA did not change the fraction or the amount of radioactivity present in plasma as verapamil at 9 minutes following verapamil administration. By 40 minutes, verapamil accounted for less than 20% of the total radioactivity. For this reason, and because differential uptake of verapamil and its metabolites into individual tissues can exist, we limited our analysis to the first 9 minutes following each verapamil injection. Over this period, at least 70% of the observed radioactivity was related to verapamil and D-617/D-717, a P-gp substrate (28). Nevertheless, the contribution of metabolites to total tissue radioactivity did not confound our estimation of P-gp inhibition because AUC ratios and the effects of CsA on tissue radioactivity, with the exception of the maternal gallbladder, were quantitatively comparable between measurements at 9, 20 and 40 minutes (see below and Fig. 6).

The shape and location of the placenta make it difficult to reliably identify and measure tracer uptake. In addition, a substantial fraction (~50%) of the placenta is maternal or fetal blood

(29). The latter is not labeled by [^{11}C]-CO. However, the fetal liver is readily identifiable on PET imaging. The substantial fetal hepatic accumulation of verapamil radioactivity served as a readily identifiable reporter of the net placental passage of verapamil into the fetal compartment (tracer concentrations in the extrahepatic fetal tissues were too low to determine their values with confidence). Although fetal movement between the PET and MRI scans complicated coregistration of PET and MR images, the exclusive concentration of radioactivity in the fetal liver made this organ readily identifiable.

In agreement with previous studies in mice and rats (2,6), the distribution of [^{11}C]-verapamil radioactivity (AUC_{0-9}) into the maternal kidneys, heart, lungs, spleen and the uterus was not significantly affected by CsA (Fig. 6). In contrast, the impact of CsA on P-gp activity was significant at the maternal BBB and the placenta. These differences in activity likely reflect higher P-gp expression in these tissues. Our interpretation of tissue P-gp inhibition is based on the assumption that the combination of verapamil and CsA results in inhibition of only P-gp. This is not an unreasonable assumption as previous studies have shown that verapamil is not a substrate of the multidrug resistance-associated protein (MRP) or breast cancer resistance protein (BCRP) and, based on its chemical structure, is unlikely to be a substrate of organic anion transporter families (5). However, we cannot exclude the possibility that in certain organs, verapamil and CsA could interact through organic cation transporters (OCTs), the organic cation transporter novel type II (OCTN2), or other, yet unidentified efflux and influx transporters (30,31).

The largest impact at the BBB was likely due to the high level of P-gp expression and function at this barrier. At the higher mean CsA blood concentration, 19.2 μM , the brain:plasma AUC ratio of [^{11}C] radioactivity changed 3.9-fold. These results are consistent with the 2.3-fold increase in cerebrum:blood AUC of [^{11}C]-verapamil radioactivity in male *M. mulatta* following the administration of the CsA analogue, PSC833 (25). In contrast, our study in rats demonstrated an approximately 12-fold increase in the brain:plasma total [^3H]-radioactivity ratio at comparable mean CsA blood concentrations (17.3 μM) (7). The lower maximal increase in the brain distribution of [^{11}C]-radioactivity in the pregnant macaques might be explained by species differences in the contribution of BBB P-gp activity to the distribution of [^3H]-verapamil radioactivity into the brain, inhibition of an influx transporter by CsA or pregnancy. However, since a similar degree of inhibition was observed in male, unanaesthetized macaques with another P-gp inhibitor, the first explanation appears to be more likely. If this conclusion is extrapolated to humans, the rodent P-gp knockout models may overestimate the potential for P-gp-mediated drug interactions at the human BBB. This may seem at odds with our previous conclusion that the rodent is an excellent predictor of the verapamil-CsA interaction at the BBB (32). However, there is no discrepancy. Although there is an excellent agreement between the interaction observed at the rat and the human BBB at the lower CsA blood concentrations, our data suggest a divergence between the rat and human as the inhibitor concentration is increased and as P-gp inhibition approaches a maximum. To test this hypothesis, additional drug interaction studies in humans with inhibitors of P-gp more potent than CsA (e.g. quinidine, itraconazole) are needed.

At the placental barrier, the significance of P-gp was demonstrated by the 2.2-fold change (122% increase) increase in fetal liver radioactivity following CsA administration. Our results indicate that CsA has a greater inhibitory effect on the efflux of verapamil across the BBB than across the placenta, likely reflecting a tighter P-gp barrier at the brain, at least for the combination of verapamil and CsA. Mean placental blood flow was not significantly affected by CsA and thus could not explain the enhanced distribution of [^{11}C]-radioactivity across the placenta. The contribution of P-gp to the placental barrier may change with gestational age. We have previously shown that P-gp expression in human placental syncytiotrophoblasts decreases ~40-fold with gestational age (10), but it is currently unknown whether this is

paralleled by a change in P-gp function. This study has demonstrated the feasibility of PET imaging pregnant non-human primates to serially measure the effect of gestational age on placental P-gp functional activity. In addition, our data suggest that P-gp-mediated drug interactions at the placental P-gp barrier are possible but their magnitude, at therapeutic concentrations of CsA, are expected to be modest. Although CsA is transferred across the placenta (33) and P-gp is expressed in many tissues of the third-trimester human fetus (34), we could not assess the effect of CsA on fetal tissue P-gp activity because the tracer concentrations in the extrahepatic fetal tissues were too low to determine their values with confidence.

In contrast to the brain and the fetus, with the exception of the gall bladder, CsA did not affect tissue distribution of [^{11}C]-radioactivity into other tissues, such as the liver and the kidneys. This is consistent with the rodent data where the contribution of P-gp at these blood-tissue barriers appears to be lesser than at the BBB and the blood-placental barrier. We speculate that the reduction in gall bladder radioactivity during CsA administration reflects the inhibition of P-gp in liver canaliculi.

Conclusion

The current study provides proof-of-concept that PET can be used in the pregnant macaque to non-invasively and simultaneously assess the impact of P-gp inhibition on distribution of drugs into multiple tissues. The inhibition of P-gp is tissue-specific, and the greatest impact is at the BBB and the placental barrier, two important barriers affecting drug distribution into the brain and the fetus respectively. In addition, the maximal inhibition of P-gp at supratherapeutic concentrations of CsA, results in lesser effect on brain distribution of [^{11}C]-verapamil radioactivity than that observed in rodent models where P-gp function is genetically or chemically ablated. If the non-human primate is representative of humans, these data suggest that genetic or chemical knock-out of P-gp in rodents will over-estimate the maximum potential of drug interactions at the human BBB when potent inhibitors of P-gp are studied. To test this hypothesis, additional drug interaction studies in humans, with inhibitors of P-gp more potent than CsA (e.g. quinidine), are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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FIGURE 1.

A schematic as well details of the PET imaging protocol. As described in Methods, the protocol for one animal was modified as well as shortened.

FIGURE 2.

Blood cyclosporine A (CsA) concentration-time profiles from the time of [^{11}C]-verapamil injection ($t=0$). The legend indicates animal number and CsA dose. The rectangle highlights the modest change in CsA blood concentration over 0-9 min., the focus of our data analysis.

*Animal was studied for only 25 minutes.

FIGURE 3.

Percent of [^{11}C]-radioactivity in plasma before and during cyclosporine A (CsA) infusion show that verapamil is rapidly metabolized in the pregnant macaque. At 9 minutes, CsA did not significantly affect the plasma radioactivity of verapamil and D-617/D-717, but decreased the radioactivity of the polar metabolites. At 40 minutes, less than 20% of the total radioactivity was verapamil in the presence or the absence of CsA. Data are expressed as mean $\pm$ SD (n=4). Data in the absence and presence of CsA (at a given time point) were compared using the Mann-Whitney test. * $P < 0.05$.

FIGURE 4.

Plasma (A) and tissues (B-L) [^{11}C]-radioactivity concentration-time profiles in a representative animal in absence (solid triangles) and presence (open triangles) of 12 mg/kg/hr cyclosporine A (CsA). In the presence of CsA, these profiles show greater distribution of radioactivity into the maternal brain (B) and the fetal liver (C), but lesser distribution into the maternal liver. In the maternal gallbladder, CsA initially increased the accumulation of [^{11}C]-radioactivity, but reduced it at later time points. CsA did not change the time-concentration profiles of radioactivity in the plasma or other tissues.

FIGURE 5.

PET images of a pregnant *M. nemestrina* before (A) and during (B) the administration of 12 mg/kg/hr cyclosporine A (CsA). The PET scans (A and B) are SUV images summed over a period of 1 to 9 minutes following [^{11}C]-verapamil injection. The images A and B were scaled to the same pixel value. (C) Image produced by pixel-by pixel subtraction of scan A from scan B. The fetal liver was the reporter of [^{11}C]-radioactivity that crossed the placental barrier. Due to P-gp inhibition, CsA significantly increased the distribution of [^{11}C]-radioactivity into the maternal brain and fetal liver (yellow to red areas in B and C). In the maternal liver, CsA decreased the amount of [^{11}C]-radioactivity (darker area in C). (D) T2-weighted MRI of the same animal. The color scale reflects SUV as shown by the thermometer.

FIGURE 6.

The effect of cyclosporine A (CsA) on the distribution (percent change in $AUC_{\text{tissue}}/AUC_{\text{plasma}}$) of $[^{11}\text{C}]$ -radioactivity into maternal brain and fetal liver was large and significant. In contrast, its effect on the distribution of $[^{11}\text{C}]$ -radioactivity into other tissues was insignificant or modest (gall bladder). Data are expressed as mean $\pm$ SD (n=4). * $P < 0.05$.

TABLE 1

Characteristics of the animals at the time of the PET study

Animal	Gestational age (days) [*]	Age (years)	Body weight (kg)	CsA dose (mg/kg/hr)
02088	159	14.5	10.3	12
02054	145	8.7	7.6	12
04052	155	12.5	8.2	24
02091	155	8.0	7.6	24
Mean	154	10.9	8.4	
SD	6	3.1	1.3	
Range	145–159	8.0–14.5	7.6–10.3	

* Gestational period of *M. nemestrina* in captivity is 172 days. CsA, cyclosporine A.

TABLE 2
The percent change in plasma or tissue distribution of [¹¹C]-radioactivity produced by cyclosporine A (CsA)

Animal Identifier	Blood CsA concentration at 5 minutes (µM) ^a	ID-normalized AUC ₀₋₉ (% change)				AUC _{tissue} :AUC _{plasma} 0-9min (% change)						
		Plasma	Maternal brain	Fetal liver	Maternal liver	Gall-bladder	Kidneys	Heart	Lungs	Spine	Spleen	Uterine wall
02088	8.2	-17.3	366.2	153.4	-0.5	76.6	36.4	45.9	45.5	31.5	23.0	22.9
02054	4.7	3.8	164.2	88.8	-12.5	-4.5	19.1	-9.3	6.2	-8.1	12.6	-20.0
04052	21.0	-22.0	322.5	35.8	-3.1	31.8	38.1	23.5	33.5	-6.7	37.8	83.6
02091	17.9	3.4	252.0	208.9	-23.7	-17.5	-7.2	6.2	-3.4	-12.7	-1.1	-16.5
Mean		-8.0	276.2 [*]	121.8 [*]	-10.0	21.6	21.6	16.6	20.5	1.0	18.1	17.5
SD		13.5	88.3	75.4	10.5	42.1	21.0	23.7	22.9	20.5	16.4	48.2

^a Approximate midpoint of the 0–9 min time period.

^{*} , *P*<0.05