Influence of functional haplotypes in the drug transporter gene \(ABCB1\) on central nervous system drug distribution in humans

**Background and Objective:** Single nucleotide polymorphisms in the human multidrug-resistance gene \(ABCB1\) have been reported to be associated with altered expression and function of P-glycoprotein, an efflux transporter, expressed at the blood-brain barrier. To test whether certain \(ABCB1\) haplotypes contribute to interindividual differences in central nervous system drug distribution, brain distribution of a model P-glycoprotein substrate, the calcium channel inhibitor verapamil, was measured by positron emission tomography (PET) in 2 groups of healthy volunteers.

**Methods:** Ten homozygous carriers (cases) of the TTT haplotype (3435T, 1236T, and 2677T) and 10 controls homozygous for the wild-type CGC haplotype (3435C, 2677G, and 1236C) were administered a mean intravenous bolus of 412 ± 114 MBq carbon 11–labeled verapamil containing less than 15 nmol of unlabeled verapamil. PET imaging of brain tissue and venous blood sampling were performed for 1 hour after dosing.

**Results:** As a measure of brain penetration, the ratio of PET area under the time-radioactivity curve (AUC) to plasma AUC was calculated from time-radioactivity curves, with a mean ratio of 1.1 ± 0.3 (SD) (95% confidence interval, 0.9-1.3) for cases and 1.1 ± 0.2 (95% confidence interval, 0.9-1.2) for controls, respectively (\(P = .96\)). Mean brain AUC values were 31.2 ± 3.9 and 35.7 ± 5.7 for the TTT and CGC haplotype, respectively (\(P = .11\)). Plasma AUCs were not significantly different.

**Conclusion:** No difference in the brain distribution of \([^{11}C]\)verapamil could be detected in healthy volunteers differing in \(ABCB1\) haplotypes. (Clin Pharmacol Ther 2005;78:182-90.)

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Several transport proteins have been shown to exert a substantial impact on the pharmacokinetics and pharmacodynamics of select drugs. In particular, the gene product of the human multidrug resistance gene \(ABCB1\) (MDR-1), P-glycoprotein, a polyfunctional transporter for a wide array of drugs including cardiac glycosides, immunosuppressive agents, human immunodeficiency virus (HIV) protease inhibitors, or tricyclic antidepressants, has received considerable attention. P-glycoprotein acts as an energy-dependent efflux pump, which translocates substrates from the intracellular to the extracellular com-
ABCB1 haplotypes and CNS distribution of $[^{11}C]$verapamil

Single nucleotide polymorphisms (SNPs) have been described in the $ABCB1$ gene, some of which were shown to influence drug distribution and clinical response to therapies with P-glycoprotein substrates. Of particular interest are mutations in exon 26 and exon 21, because these polymorphisms have been associated with differences in P-glycoprotein expression and function in humans. In preclinical experiments in knockout mice lacking P-glycoprotein, it has been shown that P-glycoprotein mutations influence the central nervous system (CNS) distribution of drugs in brain capillary endothelial cells, possibly as a result of effects at the BBB level. In those experiments, absence of functional P-glycoprotein led to a more than 20-fold increase in CNS penetration of a number of drugs, such as ivermectin, vinblastine, cyclosporine (INN, ciclosporin), digoxin, or verapamil. Recently, P-glycoprotein polymorphisms were also shown to be associated with the clinical response to antiepileptic drugs. The exact reason for this association has thus far remained elusive.

Linkage disequilibrium has recently been demonstrated between SNPs in exons 26 (C3435T), 21 (G2677T), and 12 (C1236T), suggesting that the observed differences in P-glycoprotein expression or function may be associated with polymorphisms other than the synonymous C3435T SNP. On the basis of reports that haplotype analysis is superior to unphased SNP analysis to predict $ABCB1$ phenotypes and that the TTT haplotype correlates with low P-glycoprotein activity, the aim of this study was to test whether haplotypes in the $ABCB1$ gene contribute to interindividual differences in CNS drug distribution in vivo in healthy humans.

Brain distribution of a model P-glycoprotein substrate, the calcium channel inhibitor verapamil, was measured by positron emission tomography (PET) in 2 groups of healthy volunteers with known $ABCB1$ haplotypes. PET is a noninvasive nuclear imaging technique that allows tissue concentrations of drug molecules to be measured with good spatial and temporal resolution in virtually all organs of the human body. PET has a sensitivity in the lower picomolar range but requires the drug of interest to be radiolabeled with an appropriate positron-emitting radioisotope, such as carbon 11 (half-life, 20.4 minutes) or fluorine 18 (half-life, 110 minutes). We hypothesized that the results of this study could provide evidence for the mechanisms responsible for interindividual differences in the incidence of CNS side effects or therapeutic failure of drugs designed to act in the CNS.

METHODS

The study was approved by the local ethics committee and was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice Guideline of the European Commission (EC-GCP guideline). All volunteers were given a detailed description of the study, and written consent was obtained.

Study design

The study was carried out as a descriptive, exploratory, single-center, nonrandomized, evaluator-masked, case-control study.

Healthy volunteers

One hundred one male volunteers were genotyped for $ABCB1$ SNPs C3435T, C1236T, and G2677T. For the PET study, 2 groups of 10 individuals each with the following genotypes were selected: 10 homozygous subjects (cases) with the TTT haplotype (3435T, 2677T, and 1236T) (mean age [±SD], 28 ± 4 years; mean weight [±SD], 80 ± 12 kg; mean height [±SD], 182 ± 5 cm) and 10 homozygous carriers (controls) of the CGC haplotype (3435C, 2677G, and 1236C) (mean age [±SD], 28 ± 4 years; mean weight [±SD], 77 ± 6 kg; mean height [±SD], 182 ± 6 cm).

Each volunteer was subjected to a screening examination including the following: medical history, physical examination, 12-lead electrocardiography, blood pressure, heart rate, complete blood cell count, urinalysis, urine drug screening, clinical blood chemistry evaluation, blood coagulation tests, hepatitis B surface antigen test, and HIV antibody test. Subjects were excluded if they were taking any prescription medication or over-the-counter drugs within a period of 2 weeks before the study or if they had undergone any diagnostic analysis with radioactive tracers or radiographs during the last year preceding the study.

Polymerase chain reaction analysis of $ABCB1$

Four milliliters of blood was drawn during routine venipuncture after the volunteer’s informed consent...
The use of allele-specific primers as described previously.24 Separated polymerase chain reaction was performed by use of allele-specific primers as described previously.

Study medication
One intravenous bolus containing 412 ± 114 MBq (range, 331-727 MBq) carbon 11–labeled verapamil was administered to the subjects on the study day. The specific radioactivity of [11C]verapamil at the time of injection was greater than 30 GBq/μmol, resulting in the administration of less than 15 nmol of unlabeled verapamil.

Synthesis and quality control of [11C]verapamil
Synthesis. Racemic [11C]verapamil was prepared with minor modifications as described previously.25 In brief, [11C]methyl iodide was produced in a PETtrace Mel MicroLab (GE Medical Systems, Wukesha, Wis) and then transferred into a GE TRACERlab FXC radiosynthesis module, where it was converted into [11C]methyl triflate by online passage through a heated tube (200°C) containing about 300 mg of silver triflate–impregnated graphitized carbon.26,27 [11C]Methyl triflate was trapped in a solution of racemic N-desmethyl verapamil (free base, 0.6 mg; 1.3 μmol) (Sigma-Aldrich, Deisenhofen, Germany) in 0.5 mL of acetonitrile cooled to 0°C. The reaction mixture was then heated for 2 minutes at 50°C, cooled to room temperature, diluted with 0.2 mL of acetonitrile, and subsequently injected onto a built-in semipreparative HPLC system. A Waters μBondapak C18 column (7.8 × 300 mm, 10 μm) (Waters, Milford, Mass) was eluted with a mixture of 25-mmol/L aqueous sodium dihydrogen phosphate (pH adjusted to 7.0 with 4-mol/L aqueous sodium hydroxide), acetonitrile, and methanol (39:38:23 [vol/vol/vol]) and a flow rate of 6 mL/min. On this HPLC system, N-desmethyl verapamil and [11C]verapamil eluted with retention times of 8 to 9 minutes and 11 to 12 minutes, respectively. The fraction (6-10 mL) containing [11C]verapamil was diluted with 90 mL of water and passed over a C18 Sep-Pak Plus cartridge (Waters), where the radiolabeled product was retained. The cartridge was then washed with 10 mL of water, and [11C]verapamil was eluted with 2 mL of ethanol into a glass vessel containing 9 mL of physiologic saline solution. The resulting solution was then passed through a sterile Millex-GV filter (0.22 μm) (Millipore, Bedford, Mass) into a sterile vial containing 9 mL of physiologic saline solution. By use of this synthesis procedure, 3.1 ± 1.1 GBq (n = 26) [11C]verapamil, readily formulated for intravenous administration, could be obtained in a total synthesis time of about 40 minutes, starting from 52 ± 3 GBq cyclotron-produced [11C]carbon dioxide.

Quality control. The radiochemical and chemical purity of [11C]verapamil was assessed by analytic HPLC by use of a Waters μBondapak C18 column (3.9 × 300 mm, 10 μm) eluted with 25-mmol/L aqueous sodium dihydrogen phosphate (pH 7.0)/acetonitrile/methanol (37:39:24 [vol/vol/vol]) at a flow rate of 2 mL/min (ultraviolet detection at 210 nm). The radiochemical purity of [11C]verapamil (retention time, 7-8 minutes) was greater than 99%. The content of labeling precursor N-desmethyl verapamil in the formulated product solution was less than 1 μmol/L.

Study protocol
On the study day, 2 venous catheters were placed in the subjects’ arm veins, one for infusion of radiolabeled drug and the other in the contralateral arm for blood sampling. Each subject was positioned supine on the imaging bed of the PET camera with the head in a fixing device to avoid movement artifacts. To correct for tissue attenuation of photons, a transmission scan of 10 minutes’ duration by use of two 400-MBq germanium 68 pin sources was recorded before radiotracer injection. An aliquot of the radiotracer solution was diluted with physiologic saline solution to a final volume of 20 mL and was administered as an intravenous bolus over a 10-second period. Dynamic PET imaging and venous blood sampling were initiated at the start of the intravenous bolus and were continued for 60 minutes. The following imaging frame sequence was used: 12 × 10 seconds, 6 × 30 seconds, 5 × 1 minute, 5 × 2 minutes, and 8 × 5 minutes. PET images were acquired with a GE Advance PET scanner (GE Medical Systems) with a transversal field of view of 55 cm and an axial field of view of 15 cm.

Data analysis
Reconstruction of the PET data was performed by means of iterative reconstruction with the ordered-subsets expectation maximization method with 28 subsets and 2 iterations. The loop filter (Gaussian) was set to a full width at half maximum of 4.3 mm, and a postfiltering algorithm of 6.00-mm full width at half maximum was applied. Attenuation correction was performed by use of the manufacturer’s (GE Medical Systems) segmentation algorithm for transmission data. Regions of interest (ROIs) were drawn in the reconstructed PET image that best represented brain anat-
Abbreviations

CNS = central nervous system

Results

Mean time-radioactivity curves in plasma and brain tissue after intravenous bolus administration of 412 ± 114 MBq [11C]verapamil to 20 healthy volunteers (ie, 10 carriers of ABCB1 TTT haplotype and 10 carriers of ABCB1 wild-type CGC haplotype). Results are presented as mean ± SD. SUV, Standardized uptake value.

FIG 1. Mean plasma time versus radioactivity curves after intravenous bolus administration of 412 ± 114 MBq [11C]verapamil to 20 healthy volunteers (ie, 10 carriers of ABCB1 TTT haplotype and 10 carriers of ABCB1 wild-type CGC haplotype). Results are presented as mean ± SD. SUV, Standardized uptake value.

Blood analysis

Venous blood samples (9 mL) were collected at 1, 2, 5, 10, 15, 20, 25, 30, 45, and 60 minutes after radio-tracer injection into heparinized tubes. Plasma was obtained by centrifugation at 3000 rpm for 10 minutes. Radioactivity in 1-mL aliquots of plasma was measured in a Packard Cobra II auto-gamma counter (Packard Instrument, Meriden, Conn). Radioactivity count rates (counts per minute) were converted into kilobecquerels by use of a calibration curve with known amounts of [11C]verapamil. The radioactivity concentrations (in kilobecquerels per milliliter) were decay-corrected to the time of tracer injection and normalized to the injected radiotracer amount. Because of the short physical half-life of 11C and rather low levels of radioactivity in plasma after intravenous administration of [11C]verapamil, radioactivity count rates measured in plasma aliquots were not corrected for radiolabeled metabolites.

Statistical analysis

Differences in brain and plasma radioactivity concentrations between both study groups were analyzed by use of a Wilcoxon matched pairs test with commercially available software (STATISTICA, release 5.1; StatSoft, Tulsa, Okla). P < .05 was considered significant. The study had a power of 80% to detect a difference of 20% in AUC values (α error of 5%).

RESULTS

Mean time-radioactivity curves in plasma and brain tissue after intravenous bolus administration of 412 ±
114 MBq $^{[11C]}$verapamil to 10 TTT and 10 CGC haplotype carriers are depicted in Figs 1 and 2.

After $^{[11C]}$verapamil injection, plasma radioactivity rapidly declined. Brain radioactivity uptake was rapid, and $C_{\text{max}}$ values were reached with mean $t_{\text{max}}$ values of 0.5 ± 0.3 minute and 0.6 ± 0.1 minute in subjects with $^{[11C]}$verapamil injection, plasma radioactivity rapidly declined. Brain radioactivity uptake was rapid, and $C_{\text{max}}$ values were reached with mean $t_{\text{max}}$ values of 0.5 ± 0.3 minute and 0.6 ± 0.1 minute in subjects with
the TTT haplotype and those with the CGC haplotype, respectively. Mean $C_{\text{max}}$ values (0.9 ± 0.1 [95% confidence interval (CI), 0.8-1.0] and 1.0 ± 0.2 [95% CI, 0.9-1.2]) and AUC values were not significantly different when both groups were compared ($P = .11$ for both values). Mean AUC values were 31.2 ± 3.9 (95% CI, 28.4-34.0) for the TTT haplotype and 35.7 ± 5.7 (95% CI, 31.7-39.8) for the CGC haplotype, respectively. As a measure of brain penetration, the individual ratios of AUCPET/AUCplasma were calculated (Fig 3). The mean AUCPET/AUCplasma ratio was 1.1 ± 0.3 (95% CI, 0.9-1.3) for the case group and 1.1 ± 0.2 (95% CI, 0.9-1.2) for controls ($P = .96$). The relationship between individual AUC values in plasma and brain tissue is presented in Fig 4. Plasma time-radioactivity curves were also not statistically different between both groups. Mean AUC values were 30.6 ± 9.6 (95% CI, 23.8-37.4) for the TTT haplotype and 34.2 ± 6.3 (95% CI, 29.3-39.1) for the CGC haplotype, respectively ($P = .33$); mean $C_{\text{max}}$ values were 0.9 ± 0.1 (95% CI, 1.1-3.1) for the TTT haplotype and 1.0 ± 0.2 (95% CI, 1.5-3.2) for the CGC haplotype, respectively ($P = .65$).

All study procedures were well tolerated by all volunteers. There were no serious adverse events or side effects from the administration of the study medication.

**DISCUSSION**

An increasing number of studies are addressing the association of $ABCB1$ gene polymorphisms with disposition, effects, and side effects of P-glycoprotein substrates.15,16,28,29 There is, however, still controversy as to whether, and to what extent, pharmacokinetic and pharmacodynamic properties of drugs are modified by select $ABCB1$ mutations.3 At the BBB, P-glycoprotein is expressed on the luminal surface of blood capillaries.17 It is thus hypothesized that, among other mechanisms, brain penetration of endogenous and exogenous substrates might be impeded by active P-glycoprotein–triggered efflux, which physiologically contributes to the protection of the brain but reduces, however, the clinical effectiveness of drugs used for the treatment of CNS disorders, such as HIV infections, Parkinson’s disease, brain cancer, or epilepsy. The brain might, therefore, be regarded as a sanctuary for drugs that are P-glycoprotein substrates.30

We used PET to assess the influence of 2 $ABCB1$ haplotypes in healthy volunteers on the brain distribution of the radiolabeled P-glycoprotein substrate verapamil. $[1^{14}C]$Verapamil has been developed as a PET tracer to measure P-glycoprotein function in humans. This radiotracer has been extensively evaluated in vitro and in vivo in experimental animals.12,21,22 It has been shown that the brain uptake of $[1^{14}C]$verapamil was about 10-fold higher in $mdr1$ knockout mice as compared with wild-type mice.12 Moreover, pretreatment of rats with the P-glycoprotein modulator cyclosporine resulted in a 13-fold increase in brain concentrations of $[1^{14}C]$verapamil as compared with untreated animals.21 Both findings indicate that the brain distribution of $[1^{14}C]$verapamil is influenced by P-glycoprotein function. Similar results have previously been described in 2 human studies.31,32 In healthy volunteers cyclosporine-mediated P-glycoprotein inhibition resulted in a significant 88% increase in the AUCbrain/AUCplasma ratio of $^{11}C$ radioactivity.31 Administration of cyclosporine or tacrolimus to 4 lung transplant patients undergoing immunosuppressant therapy was shown to decrease $[1^{14}C]$verapamil efflux from the brain as compared with a control group of healthy volunteers.32

The current study in healthy volunteers demonstrated rapid brain uptake and slow washout of radioactivity after intravenous bolus administration of approximately 400 MBq $[1^{14}C]$verapamil containing a tracer dose of a few micrograms of unlabeled drug. Similar pharmacokinetics have been reported for $[1^{14}C]$verapamil in tumor-bearing rats and the brains of $mdr1$ knockout mice.12,30 The rather low brain uptake of $[1^{14}C]$verapamil (SUV <1) might be explained by a high degree of plasma protein binding of verapamil (85%-90%), which is known from the literature.33 The slow brain washout of the radiotracer might be the result of nonspecific $[1^{14}C]$verapamil binding in the brain as a result of its high lipophilicity (log $P = 3.8$).21,22 Previous studies have demonstrated that after injection of $[1^{14}C]$verapamil to mice, more than 90% of the total radioactivity in the brain was present as parent $[1^{14}C]$verapamil 1 hour after drug injection, which suggests that radiolabeled metabolites of $[1^{14}C]$verapamil had practically no influence on the measurement of verapamil brain concentrations with PET.12

As a measure of brain penetration of $[1^{14}C]$verapamil, AUCPET/AUCplasma ratios (Fig 3) were calculated from AUCs that were generated from individual time-radioactivity curves. AUCPET/AUCplasma ratios were not different between the 2 volunteer groups; however, high interindividual variations were observed, with a range of 0.8 to 1.5. Overall, subjects with the TTT haplotype did not differ from those with the CGC haplotype when demographic factors, smoking and drinking habits, drug intake, or previous participation in drug trials was compared. On the basis of previous data from animal models and humans, an association between subject genotype and phenotype (ie, modified brain penetration of vera-
for MRP1, it might be speculated that molecules other than P-glycoprotein transport across the BBB or P-glycoprotein–mediated BBB transport was investigated in knockout mice with a completely disrupted mdr1a gene or after pharmacologic challenge of the transporter molecule. Although the knockout experiments provided valuable mechanistic information on the contribution of P-glycoprotein to CNS distribution, they failed to reflect a less extreme in vivo situation in humans. In healthy subjects P-glycoprotein expression might be variable because of a lack of up-regulation of the ABCB1 gene in response to cellular stress triggered by drugs, toxins, or environmental factors. In patients, on the other hand, transporter expression may be markedly increased, which has been demonstrated for patients with medically intractable epilepsy, although it still remains unclear whether ABCB1 gene expression in these patients is innate or acquired.

It thus remains to be demonstrated whether, and to what extent, drug resistance at the BBB level can be attributed to polymorphisms of the ABCB1 gene. Besides P-glycoprotein, other members of the multidrug resistance–associated (MRP) transporter family are also expressed in the BBB and have substrate specificity that overlaps that of P-glycoprotein. Whereas [11C]verapamil has been shown to be a relatively poor substrate for MRP1, it might be speculated that molecules other than P-glycoprotein or MRP1 might contribute to BBB transport of [11C]verapamil. We, therefore, hypothesize that the observed interindividual differences in the brain penetration of [11C]verapamil in cases and control subjects reflect experimental variability or other, as yet unknown variables, whereas genetic variants of the ABCB1 gene had no effect on BBB penetration. Our findings are also underlined by an increasing number of publications that have failed to show an effect of ABCB1 SNPs on P-glycoprotein transporter function and drug disposition.

In conclusion, this study did not detect a statistically significant difference in the brain distribution of [11C]verapamil in healthy volunteers differing in ABCB1 haplotypes.

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