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Investigation of the Metabolites of (S,S)-[¹¹C] MeNER in Humans, Monkeys and Rats

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Abstract

Introduction—(S,S)-[¹¹C]MeNER ((S,S)-2-(α-(2-[¹¹C]methoxyphenoxy)benzyl)morpholine) is a positron emission tomography (PET) radioligand recently applied in clinical studies of norepinephrine transporters (NETs) in the human brain *in vivo*. In view of further assessment of the suitability of (S,S)-[¹¹C]MeNER as a NET radioligand, its metabolism and the identity of the *in vivo* radiometabolites of (S,S)-[¹¹C]MeNER are of great interest.

Materials and Methods—Thus, PET studies were used to measure brain dynamics of (S,S)-[¹¹C]MeNER, and plasma reverse-phase radiochromatographic analysis was performed to monitor and quantify its rate of metabolism. Eighteen healthy human volunteers, five cynomolgus monkeys, and five rats were studied.

Results and Discussion—In human subjects, the plasma radioactivity representing (S,S)-[¹¹C]MeNER decreased from 88±5% at 4 min after injection to 82±7% at 40 min, while a polar radiometabolite increased from 3±3% to 16±7% at the same time-points, respectively. A more lipophilic radiometabolite than (S,S)-[¹¹C]MeNER decreased from 9±5% at 4 min to 1±2% at 40 min. In monkeys, plasma radioactivity representing (S,S)-[¹¹C]MeNER decreased from 97±2% at 4 min to 74±7% at 45 min, with a polar fraction as the major radiometabolite. A more lipophilic radiometabolite than (S,S)-[¹¹C]MeNER, constituted 3±2% of radioactivity at 4 min and was not detectable later on. In rats, 17±4% of plasma radioactivity was parent radioligand at 30 min with the remainder comprising mainly a polar radiometabolite. (S,S)-[¹¹C]MeNER in rat brain and urine at 30 min after injection were 90% and 4%, respectively. On a brain regional level, parent radioligand ranged from 87.5±3.9% (57.2±14.2% SUV [standard uptake values, % injected radioactivity per mL multiplied with animal weight (in g)]; cerebellum) to 92.9±1.8% (36.1±4.7% SUV; striatum), with differential distribution of the radiometabolite in the cerebellum (6.7±0.3% SUV) and the striatum (2.5±0.3% SUV). Liquid chromatography-mass spectrometry analysis of rat urine identified a hydroxylation product of the methoxyphenoxy ring of (S,S)-MeNER as the main metabolite. In the brain, the corresponding main metabolite was the product from *O*-de-methylation of (S,S)-MeNER. PET measurements were performed in rats as well as in wild-type and P-gp-knock-out mice. In rats, the brain peak level of radioactivity was found to be very low (65% SUV). In mice, there was only a small difference in peak brain accumulation between P-gp knock-out and wild-type mice (145 vs.

125%SUV) with the following rank order of regional brain radioactivity: cerebellum $\times$ thalamus > cortical regions > striatum.

Conclusion—It can be concluded that radiometabolites of (*S,S*)-[^{11}C]MeNER are of minor importance in rat and monkey brain imaging. The presence of a transient lipophilic radiometabolite in peripheral human plasma may induce complications with brain imaging, but its kinetics appear favorable in relation to the slow kinetics of (*S,S*)-[^{11}C]MeNER in humans.

Keywords

MeNER; Metabolism; PET

Introduction

The vast majority of drugs undergo metabolism in vivo. Likewise, positron emission tomography (PET) radioligands are metabolized, yielding radiolabeled products (radiometabolites) that may have great importance in the biomathematical modeling of their respective biological targets [1]. Not only may the extent of radioligand metabolism be useful to assess, but also the chemical nature of radiometabolites and their biodistributions. Especially in brain receptor imaging, it is of high importance that radiometabolites do not pass the blood–brain barrier (BBB), as PET does not distinguish between radiochemical entities. Thus, a significant risk of obtaining confounded images is present in all PET measurements with novel radioligands.

PET measurements with the 5-HT_{1A} receptor radioligand, [^{11}C]WAY 100635, explicitly exemplify the importance of performing radiometabolite analysis. In this case, the problem with a BBB-permeable metabolite was elegantly solved by changing the position of the label in the radioligand [2].

(*S,S*)-[^{11}C]MeNER has been considered a candidate radioligand for imaging brain norepinephrine transporters (NETs) in the living human brain with PET [3–5]. As part of further examination of the suitability of (*S,S*)-[^{11}C]MeNER for imaging NETs in vivo by PET, we were interested in assessing the metabolism of this radioligand.

MeNER is an *O*-methyl homolog of the antidepressant, reboxetine (Edronax®), whose metabolism has been extensively reviewed by Dostert et al. [6]. The pharmacokinetics of reboxetine is significantly faster in rats and monkeys than in humans, with plasma half-lives of 0.9, 1.6, and 12.5 h, respectively. Reboxetine undergoes extensive oxidative metabolism in all species, through three major metabolic pathways: (1) hydroxylation of the ethoxy-bearing aryl ring, (2) *O*-de-ethylation, and (3) oxidation of the morpholinyl moiety [6]. Due to the close structural similarity between MeNER and reboxetine, we expect the biotransformation of (*S,S*)-[^{11}C]MeNER to be similar to that of reboxetine, as depicted in Scheme 1.

The aim of this study was to assess the extent and nature of the metabolism of (*S,S*)-[^{11}C]MeNER in human, monkey, and rat plasma with a view to understanding their possible implications for brain imaging with PET.

Subjects, Materials and Methods

Analysis of Radiometabolites in Human Plasma

Eighteen healthy human volunteers were examined at the Department of Clinical Neuroscience at the Karolinska Hospital in Stockholm after approval by the Ethics and the Radiation Safety Committees of the Karolinska Hospital and the Medical Products Agency of Sweden. The study was guided by the ALARA (“as low as reasonable achievable”) principle for radiation

exposure and performed in accordance with the ethical principles provided by the Declaration of Helsinki, consistent with Good Clinical Practice and applicable regulatory requirements.

A reverse-phase high-performance liquid chromatography (HPLC) method was used to determine the percentages of radioactivity in human plasma that correspond to unchanged radioligand and radiometabolites during the course of a PET measurement [7]. Arterial blood samples (2 mL) were obtained at 4, 10, 20, 30, and 40 min after injection of (*S,S*)-[¹¹C]MeNER. Plasma (0.5 mL) obtained after centrifugation of blood at 2,000×*g* for 2 min was mixed with acetonitrile (0.7 mL). The supernatant acetonitrile–plasma mixture (1.1 mL) and the precipitate obtained after centrifugation at 2,000×*g* for 2 min were counted in a NaI well counter.

The radio–HPLC system used in the plasma experiments consisted of an interface module (D-7000; Hitachi), a L-7100 pump (Hitachi), an injector (model 7125 with a 1.0 mL loop; Rheodyne) equipped with a μ -Bondapak-C18 column (300×7.8 mm, 10 μ m; Waters), and an absorbance detector (L-7400; 254 nm; Hitachi) in series with a radiation detector (Radiomatic 150TR; Packard) equipped with a PET Flow Cell (600 μ L cell). Phosphoric acid (10 mM) (A) and acetonitrile (B) were used as the mobile phase at 6.0 mL/min, according to the following program: 0–5.5 min, (A/B) 90:10 $\rightarrow$ 40:60 *v/v*; 5.5–6.5 min, (A/B) 40:60 $\rightarrow$ 90:10 *v/v*; 6.5–10 min, (A/B) 90:10 *v/v*. Peaks for radioactive compounds eluting from the column were integrated and their areas expressed as a percentage of the sum of the areas of all detected radioactive compounds (decay-corrected).

Analysis of Radiometabolites in Monkey Plasma

Five cynomolgus monkeys were supplied by the Astrid Fagraeus Laboratory (Solna, Sweden). The study was approved by the Animal Ethics Committee of Northern Stockholm. Anesthesia was induced and maintained throughout the PET measurements by repeated intramuscular injection of a mixture of ketamine (3–4 mg kg^{−1} h^{−1} Ketalar®, Parke-Davis) and xylazine hydrochloride (1–2 mg kg^{−1} h^{−1} Rompun® vet., Bayer Sweden).

Venous blood samples (2 mL) were obtained at 4, 15, 30, and 45 min after injection of (*S,S*)-[¹¹C]MeNER and were treated in a similar manner to that described for the analysis of radiometabolites in human plasma (vide supra).

Analysis of Radiometabolites in Rat Brain, Plasma, and Urine

Five male Sprague–Dawley rats weighing between 353 and 384 g were anesthetized with 1.5% isoflurane in oxygen. (*S,S*)-[¹¹C]MeNER in saline (1.7±0.3 mCi; 0.66±0.1 μ g carrier, (1.8±0.3 μ g/kg)); specific radioactivity (SR; 783±100 mCi/ μ mol; *n*=3) was then administered via the penile vein to three rats (368±8 g) for ex vivo analysis and to two rats of 353 and 398 g [1.1 mCi; carrier 0.44 μ g (1.25 μ g/kg); SR 712 mCi/ μ mol, and 1.3 mCi; carrier 0.38 μ g (0.94 μ g/kg); SA 1,011 mCi/ μ mol] for PET imaging studies. Thirty minutes later, urine was aspirated through a syringe and a hypodermic needle directly from the urinary bladder, and anti-coagulated (heparinized) blood was collected by cardiac puncture. Then, the rat was killed by increased anesthesia and injection of a saturated potassium chloride solution followed by decapitation. Regional brain sections [hypothalamus, striatum, cerebellum, and the remainder of the brain (R-brain)] were resected, weighed, and counted in a γ -counter (counting efficiency=51.84%) and then homogenized in 1.5× their volume of MeCN (spiked with MeNER) followed by the addition of water (100 μ L) and repeated homogenization. After each tissue homogenization, the homogenizer was washed by three consecutive sham homogenizations in clean acetonitrile to reduce cross contaminations. The homogenate was finally centrifuged at 10,000×*g* for 10 min, and the supernatant was collected for analysis. Each rat plasma sample (450 μ L, separated from blood by centrifugation) was added to a prespiked solution of authentic MeNER in acetonitrile (700 μ L) and thoroughly mixed. Water (100 μ L)

was added and the suspension mixed again, counted in a γ -counter for total radioactivity and centrifuged at $10,000\times g$ for 1 min. Radioactivities in the resulting precipitates were used to calculate the percent recovery of radioactivity in the acetonitrile supernates. The clear prefiltered supernatant liquids were analyzed simultaneously by radio-HPLC on two separate systems.

The first system (basic system) employed a Novapak® C₁₈ column (Waters, Milford, MA, USA) with a radial compression module RCM-100, eluted with methanol–water–triethylamine (70:30:0.1 by vol.) at 2.0 mL/min. Eluate was monitored with an in-line flow-through Na(Tl) scintillation detector (Bioscan). Chromatographic data were corrected for physical decay, stored, and analyzed by “Bio-Chrom Lite” software (Bioscan).

The second system (acidic system) utilized a μ -Bondapak C₁₈ column (300 $\times$ 3.9 mm, 10 μ m; Waters) eluted at 3 mL/min with the same gradient as employed in the analysis of radiometabolites in human and monkey plasma (vide supra). In this case, 1.5 mL fractions were collected from the column outlet, measured in the γ -counter, and plotted vs. time to obtain the chromatograms.

One rat (384 g) was constantly infused (CI) intravenously (via penile vein) with low SR (S,S)-[¹¹C]MeNER [8.7 mCi; carrier 1.0 mg, (2.6 mg/kg); SA 2.59 mCi/ μ mol] for 1 h. Blood, urine and brain tissues were collected in a similar manner as described above.

Percent SUVs (%standard uptake values) were calculated from radioactivity concentrations (% injected radioactivity per mL) by multiplying with the mass of the animal (in g).

Analysis of Metabolites in Low Specific Radioactivity Experiments by LC-MS

Rat brain extracts (in acetonitrile) were thawed, and 1 mL of each was concentrated with a SpeedVac evaporator (Thermo Electron Corp.; Milford, MA, USA). Each residue was reconstituted in aqueous methanol (50%; 200 μ L) and centrifuged ($5,000\times g$; 5 min). The clear extract was transferred to an autosampler vial for liquid chromatography-mass spectrometry (LC-MS) analysis. Thawed rat urine samples were centrifuged and the supernates injected into LC-MS.

An LCQ Deca model LC-MS from Thermo Electron Corporation (San Jose, CA, USA) was used for MS experiments. The HPLC mobile phase consists of 10 mM ammonium formate solution in water (C) and a mixture (D) of acetonitrile and water (90:10 v/v) containing 10 mM ammonium formate. The HPLC pump ran the following gradient: 0–2 min, (C/D) 80:20, flow 150 μ L/min; 2–10 min, (C/D) 80:20 $\rightarrow$ 20:80 v/v, flow 150 μ L/min; 10–16 min, (C/D) 20:80 v/v, 250 μ L/min; 16–17 min, (C/D) 20:80 $\rightarrow$ 80:20 v/v, 250 μ L/min; 17–20 min, (C/D) 80:20 v/v, 250 μ L/min. Reverse-phase HPLC analysis was carried out using a Phenomenex (Torrance, CA, USA) column, Synergi Fusion-RP column (4 μ m, 150 $\times$ 2 mm).

The prepared brain extracts (10 μ L), urine samples (2 μ L), and standard MeNER solution (1 μ L; 10 ng/mL) in 50% aq. MeCN were injected into the LC-MS. At 2.5 min after injection, the HPLC flow was diverted from waste to the electrospray module of the LC-MS for electrospray ionization. Settings were: spray voltage 5.0 kV, sheath gas (N₂) flow rate 65 U, auxiliary gas flow rate 10 U, capillary voltage 38 V, and capillary temperature 260°C. In the full scan mass spectrometry (MS) acquisition, the instrument method was set up to detect ions ranging between *m/z* values 150 and 600. In tandem mass spectrometry (MS-MS) experiments, the molecular ion of MeNER or its metabolite was isolated with 1.5 amu width and dissociated in the ion trap at a collision energy level of 30%.

Control tissue samples, from rats that received no treatment, were similarly analyzed to confirm the absence of metabolite-specific ions. Verification of chemical structures by MS-MS experiments were performed for each metabolite by comparing the daughter-ion spectrum with that of MeNER.

PET Measurements in Healthy Human Volunteers

PET imaging studies were performed in six healthy human volunteers. The PET experimental procedure that was employed in this work has been previously described [8].

PET Measurements in Rats and Mice

PET imaging studies were performed in two rats. Rats were anesthetized by inhalation of 1.5% isoflurane in oxygen and were injected with a bolus of (S,S)-[¹¹C]MeNER. Rat PET brain imaging studies were carried out with an Advanced Technology Laboratory Animal Scanner (ATLAS; NIH). Serial dynamic image data acquisition began at the time of injection and continued for 1.5 h period after injection, with a frame schedule of 20 s×6, 60 s×5, 120 s×4, 300 s×3, 600 s×3.

Male P-gp knock-out mice [*mdr-1a/1b*(-/-)] [9] (model; 001487-MM, double homozygotes) or wild type [*mdr-1a/1b*(+/+)] (Model; FVB) were purchased from Taconic, Germantown, NY, USA. One knock-out, and one wild-type mouse were each anesthetized with 1.5% isoflurane and injected simultaneously with (S,S)-[¹¹C]MeNER, 708.9 and 510.7 μCi, respectively. Mice were both placed in the PET camera gantry, and imaging studies were carried out with the ATLAS (NIH). Serial dynamic image data were acquired according to the same method described for the rat PET study (vide supra).

Chemistry

Radiochemistry—(S,S)-[¹¹C]MeNER was prepared as previously described [3,4]. The precursor for radiolabeling, desethyl-reboxetine, and standard ((S,S)-MeNER) were obtained from Eli Lilly Co. (IN, USA). Solid phase extraction columns (C-18 Sep-Paks) were obtained from Waters. Other chemicals were obtained from commercial sources and were of analytical grade and used without further purification.

2-(α-(2-Methoxyphenoxy)benzyl)N-methyl-morpholine (4)—To a solution of racemic MeNER (10 mg, 0.13 mmol) in dimethylformamide (0.4 mL) was added triethylamine (27 mg, 0.27 mmol) and methyl iodide (38 mg, 0.27 mmol). The solution was stirred overnight, after which the reaction mixture was diluted with water and passed through a C18 Sep-Pak. The Sep-Pak was washed with water and then eluted with water–acetonitrile (3:1 v/v). The fraction containing the title compound was collected and used as a reference solution for chromatography; LC-MS, single component, *t*_R 2.7 min, relative abundance: 314.0 (100%), 314.9 (20%).

Results

Analysis of Radiometabolites in Human Plasma

The recovery of radioactive material from pellets was >85%, and the recoveries from the HPLC column were 92±7% (*n*=7). The plasma radioactivity represented by unchanged (S,S)-[¹¹C] MeNER (Fig. 1, fraction II; retention time (*t*_R) 5.7 min) in the human subjects decreased from 88±5% at 4 min after injection to 82±7% at 40 min (Fig. 1). A more hydrophilic radiometabolite (fraction I) was eluted unretained from the HPLC column and increased from 3±3% at 4 min to 16±7% of plasma radioactivity at 40 min. A more lipophilic radiometabolite, fraction III (*t*_R 7.5 min), decreased from 9±5% at 4 min to 1±2% at 40 min.

Analysis of Radiometabolites in Monkey Plasma

The plasma radioactivity represented by (*S,S*)-[¹¹C]MeNER in monkeys decreased from 97±2 at 4 min to 74±7% at 45 min, with the polar fraction I as the major radiometabolite. Fraction III was 3±2% of radioactivity at 4 min and decreased to such low concentrations that it was undetectable and consequently unquantifiable at later time-points.

Analysis of Radiometabolites in Rat Brain, Plasma, and Urine

The recovery of radioactive material from pellets and from the HPLC system were both greater than 97%.

One major radiometabolite fraction was found in rat brain and plasma, eluting unretained from the HPLC column under both acidic and basic analytical conditions. This comprised about 10% and 70% of the total brain and plasma radioactivity, respectively.

In urine, a second radiometabolite was observed [*t_R* 3.0 (basic) or 4.5 min (acidic)], which was not observed in rat brain or plasma, eluting between the parent [*t_R* 4.8 (basic) or 5.5 min (acidic)] and the unretained hydrophilic radiometabolite fraction observed in brain and plasma. This radiometabolite comprised about 35% of total urine radioactivity, with the remainder being mainly highly hydrophilic species (60%) and a small portion of parent radioligand (4%).

On a brain regional level, the radiometabolite concentrations were lower than in the plasma (Fig. 2). The regional brain distribution of radiometabolites of (*S,S*)-[¹¹C]MeNER was heterogenous, with higher accumulation in extrastriatal brain regions. Ratios of radiometabolite concentration in the various tissues to that in the striatal tissue were highest for the cerebellum (2.8±0.1, *n*=3) followed by the rest of brain (2.4±0.3, *n*=3) and the hypothalamus (2.2±1.5, *n*=3).

In the carrier-added experiment, low amounts of (*S,S*)-[¹¹C]MeNER entered the rat brain (65% SUV).

PET Measurements in Healthy Human Volunteers

After injection of (*S,S*)-[¹¹C]MeNER into healthy human volunteers, radioactivity accumulated in the pulvinar, which is a subregion of thalamus (Fig. 3). Lower accumulation was observed in the caudate, with a maximum ratio of radioactivity between the pulvinar and caudate of 1.5 at 72 min after injection. The radioactivity was cleared rapidly from plasma (Fig. 3).

PET Measurements in Rats and Mice

At 30 min after injection of (*S,S*)-[¹¹C]MeNER, the brain radioactivities (70–80% SUV) as measured with PET in two rats, were similar to those measured by ex vivo analysis (i.e., about 65% SUV). The regional distribution of radioactivity in rat brain was not examined due to low global brain radioactivity accumulation.

In the wild-type mouse, peak brain radioactivity (125% SUV) was higher than that of rats. Accumulation of radioactivity in the brain of a P-gp-knock-out mouse (145% SUV) was slightly higher than in that of a wild-type (125% SUV). The radioactivity accumulation on a brain regional level was highest in cerebellum and thalamus followed by intermediate levels in the cortical regions. The lowest accumulation of radioactivity was observed in striatum.

Analysis of Metabolites for Low Specific Radioactivity Injections

In MS-MS analysis of MeNER, the daughter ion spectrum showed *m/z* 176 ion as the dominant ion. Other minor fragment ions in this spectrum are *m/z* 158 and *m/z* 131.

In the LC-MS analysis of rat brain extract, the reconstructed ion-chromatogram between m/z values 285 and 301 showed two peaks, a minor metabolite peak at t_R 9.66 min and un-metabolized MeNER at t_R 10.29 min. The retention time and MS and MS-MS data for MeNER in the brain extract matched those of authentic MeNER. The mass spectrum for the peak at t_R 9.66 min showed molecular ion m/z 286 and a fragment ion m/z 176. These ions are characteristic of the metabolite desmethyl-MeNER (**5**, Scheme 1). Additionally, MS-MS analysis of this metabolite showed fragment ions m/z 176, m/z 158, and m/z 131 consistent with this metabolite being desmethyl-MeNER.

In rat urine, the ion chromatogram for m/z values between 299 and 317 showed two peaks, one metabolite at t_R 8.16 min and MeNER at t_R 10.21 min. The mass spectrum for unmetabolized MeNER in urine showed the molecular ion m/z 300 and the characteristic fragment ion m/z 176. The mass spectrum of the metabolite peak showed a molecular ion m/z 316 assignable to a hydroxylated derivative of MeNER (**2**, Scheme 1). There was no mass shift in the fragment ion, m/z 176, indicating that hydroxylation occurred on the methoxyphenyl moiety of MeNER. MS-MS analysis of the molecular ion, m/z 316 generated the dominant fragment ion m/z 176, consistent with the proposed position of the hydroxyl group in the metabolite. LC-MS analysis detected no other significant metabolites.

Discussion

Radiometabolite analysis is a crucial step in radioligand development. In this report, the radiometabolism of (S,S)-[^{11}C]MeNER, a NET radioligand, was evaluated in rats, monkeys, and humans.

Consistent with a previous report on the metabolism of (S,S)-[^{11}C]MeNER in rats, a small fraction of the radioactivity in brain, about 10% (vs. < 5%), was found to correspond to radiometabolites [4]. Although we observed one radiometabolite in rat brain, it was not present at a level that would cause serious concern for imaging studies. Furthermore, the major metabolite found in rat brain extracts in a carrier-added experiment with (S,S)-MeNER was *O*-demethylated MeNER. In PET experiments with (S,S)-[^{11}C] MeNER, this type of metabolism is ideal, since *O*-demethylation of (S,S)-[^{11}C]MeNER would result in an unlabeled metabolite that is unable to confound PET images. Curiously, the radiometabolite that was observed in rat brain seemed to have some target selectivity, but the structure of this radiometabolite was not elucidated in LC-MS analyses of the rat brain extracts.

Our analysis of rat plasma showed only 17% of radioactivity represented by parent radioligand at 30 min compared to the value of 50% parent at 30 min previously reported by Wilson et al. [4]. The rats in the present study demonstrated higher rate of metabolism than those reported by Wilson et al. [4]. Difference in the observed in vivo rate of metabolism of (S,S)-[^{11}C] MeNER may include the fact that the rats used in Wilson's study were kept on a reverse 12-h light/12-h dark cycle thus adding to their slow metabolism, while our rats were not. Another possible reason for the observed differences is that our rats were anesthetized while Wilson's rats were not.

A small amount of (S,S)-[^{11}C]MeNER (2.5% SUV) was found to be excreted in the urine. Consistently, in metabolite analyses of the *O*-ethyl homolog, reboxetine, less than 0.03% of injected dose was found in the urine [6]. In a low specific radioactivity experiment, we identified the major radiometabolite in rat urine to be a hydroxylation product of the methoxy-phenoxy moiety of MeNER (Scheme 1). In previous metabolite studies with [^{14}C]reboxetine, no metabolite was found to be predominant in rat urine. The differences between these results may arise from the method of administration. In previous metabolite studies on reboxetine, [^{14}C]reboxetine was given orally, whereas we administered (S,S)-[^{11}C]MeNER intravenously.

Also, although unlikely, it cannot be excluded that MeNER and reboxetine are metabolized by different pathways.

A reason for the unexpectedly low radioactivity accumulation in rat brain (65% SUV) may be that (*S,S*)-[¹¹C]MeNER acts as a substrate for brain efflux pumps, such as P-glycoprotein (P-gp) [9]. However, the accumulation of radioactivity in brains of mice was not largely dependent on the expression of P-gp, as verified with an experiment with a P-gp knock-out mouse. Therefore, in this case, another unidentified species-dependent mechanism may be operating. It may be noted that the twofold higher accumulation of radioactivity in mouse brain than in rat brain, is consistent with previous reports on the accumulation of [¹⁴C]reboxetine in rat and mouse brain [6].

In cynomolgus monkey plasma, we found that the major radiometabolite of (*S,S*)-[¹¹C]MeNER was highly hydrophilic as observed in baboon by Ding et al. [5]. We also observed a more than twofold slower rate of metabolism in our study (74% parent at 45 min in cynomolgus monkey compared to 30% parent at 30 min in baboon) [5]. This discrepancy may be due to differing degrees of anesthesia between the two studies. We used repeated intramuscular injection of a mixture of ketamine (3–4 mg kg⁻¹ h⁻¹) and xylazine (1–2 mg kg⁻¹ h⁻¹), whereas Ding et al. used a single intramuscular injection of 10 mg kg⁻¹ and then maintained anesthesia with oxygen, nitrous oxide, and isoflurane. It has been shown that ketamine decreases the rate of de-methylation of *O*-ethyl-morphine in rats in vivo by interacting with CYP3A [10], the same complex principally responsible for the metabolism of reboxetine [11]. The slower metabolic rate observed in our study may be explained, at least in part, by inhibition of CYP3A with ketamine. Species differences between cynomolgus monkeys and baboons may also play a role.

In conscious human subjects, metabolism was as slow as in monkeys, with 82% of radioactivity represented by parent radioligand in plasma at 40 min. In early plasma samples, a lipophilic radiometabolite was observed, corresponding to about 9% of the total plasma radioactivity at 4 min and about 1% at 40 min. The same radiometabolite was also observed in monkey plasma, but was much less prevalent (3%) and only present in the first sample at 4 min. Based on the retention time of this radiometabolite in radio-HPLC, it is more lipophilic than (*S,S*)-[¹¹C]MeNER itself. It is thus likely that this radiometabolite crosses the BBB [12,13]. The impact of this radiometabolite is however hard to determine, as it is transient and almost totally cleared (1±2%) from plasma at 40 min after injection. Most likely, it is cleared from the brain at a similar rate as it is from plasma and hence does not impose any complication on the measurements of binding potentials, which are estimated at 63–93 min after injection [14]. Another possibility, although less likely, is that the radiometabolite is formed in the periphery and then transported into the brain, where it is trapped. However, this would require that a highly hydrophilic species (e.g., an acid metabolite) that is unable to cross the BBB would be generated inside the brain. The latter scenario is unlikely, but not impossible, as it has been shown that an ester moiety can be used to increase the BBB permeability of an acid radioligand in a “pro-drug” manner [15].

After injection of (*S,S*)-[¹¹C]MeNER into healthy human volunteers, radioactivity accumulated in the NET-rich thalamus, with a radioactivity ratio of about 1.5 between pulvinar and the NET-poor caudate [16–18]. A drawback with (*S,S*)-[¹¹C]MeNER is its slow kinetics in the human brain, which is obvious when viewing the rather flat time–activity curves (Fig. 2 and Fig. 3). Zoghbi et al. showed that BBB permeable radiometabolites may be discovered by analyzing differences in time-dependent changes in distribution volumes [19]. In the report by Zoghbi et al., the distribution volume of the nontarget region was increasing with time, whereas the distribution volume of the target region was slightly decreasing, suggesting a nonspecific accumulation of the radiometabolite. In the case with (*S,S*)-[¹¹C]MeNER, the

distribution volume is rather flat for the nontarget region and slightly increasing for the target region (data not shown). This may either be explained by the slow kinetics of (S,S)-[¹¹C] MeNER or specific accumulation of a radiometabolite.

In rats, we were unable to detect any radiometabolites that were more lipophilic than parent radioligand. We observed a species dependency in the rate of metabolism of MeNER, with rate of metabolism ascending in the order humans, monkeys, rats, as found with reboxetine [6]. This may explain the absence of transiently detectable lipophilic radiometabolite in rat.

In conclusion, we have performed a comparative analysis of the metabolism of (S,S)-[¹¹C] MeNER in rats, monkeys, and humans. Our findings imply that radiometabolites will have insignificant or no effect on brain PET imaging in rats and monkeys. In human subjects, significant amounts of a transient lipophilic metabolite were detected in plasma. The kinetics of this radiometabolite in plasma appears favorable to successful PET imaging, as this radiometabolite is rapidly cleared.

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References

1. Maziere, B.; Cantineau, R.; Coenen, HH.; Guillaume, M.; Halldin, C.; Luxen, A.; Loc'h, C.; Luthra, SK. Radiopharmaceuticals for positron emission tomography—methodological aspects. Stöcklin, G.; Pike, VW., editors. Dordrecht, The Netherlands: Kluwer Academic Press; 1993.
2. Osman S, Lundkvist C, Pike VW, Halldin C, McCarron JA, Swahn CG, Farde L, Ginovart N, Luthra SK, Gunn RN, Bench CJ, Sargent PA, Grasby PM. Characterisation of the appearance of radioactive metabolites in monkey and human plasma from the 5-HT1A receptor radioligand, [carbonyl-¹¹C] WAY-100635—explanation of high signal contrast in PET and an aid to biomathematical modelling. *Nucl Med Biol* 1998;25:215–223. [PubMed: 9620626]
3. Schou M, Halldin C, Sovago J, Pike VW, Gulyas B, Mozley PD, Johnson DP, Hall H, Innis RB, Farde L. Specific in vivo binding to the norepinephrine transporter demonstrated with the PET radio-ligand, (S,S)-[¹¹C]MeNER. *Nucl Med Biol* 2003;30:707–714. [PubMed: 14499328]
4. Wilson A, Johnson D, Mozley P, Hussey D, Ginovart N, Nobrega J, Garcia A, Meyer J, Houle S. Synthesis and in vivo evaluation of novel radiotracers for the in vivo imaging of the norepinephrine transporter. *Nucl Med Biol* 2003;30:85–92. [PubMed: 12623106]
5. Ding YS, Lin KS, Garza V, Carter P, Alexoff D, Logan J, Shea C, Xu Y, King P. Evaluation of a new norepinephrine transporter PET ligand in baboons, both in brain and peripheral organs. *Synapse* 2003;50:345–352. [PubMed: 14556239]
6. Dostert P, Benedetti MS, Poggesi I. Review of the pharmacokinetics and metabolism of reboxetine, a selective noradrenaline reuptake inhibitor. *Eur Neuropsychopharmacol* 1997;7:S23–S35. [PubMed: 9169308]discussion S71–3
7. Halldin, C.; Swahn, C-G.; Farde, L.; Sedvall, G. Radioligand disposition and metabolism. In: Comar, D., editor. PET for drug development and evaluation. Dordrecht: Kluwer Academic Publishers; 1995. p. 55-65.
8. Lundberg J, Odano I, Olsson H, Halldin C, Farde L. Quantification of ¹¹C-MADAM binding to the serotonin transporter in the human brain. *J Nucl Med* 2005;46:1505–1515. [PubMed: 16157534]
9. Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, te Riele HP, et al. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. *Cell* 1994;77:491–502. [PubMed: 7910522]

10. Meneguz A, Fortuna S, Lorenzini P, Volpe MT. Influence of urethane and ketamine on rat hepatic cytochrome *P*450 in vivo. *Exp Toxicol Pathol* 1991;51:392–396. [PubMed: 10445403]
11. Wienkers LC, Allievi C, Hauer MJ, Wynalda MA. Cytochrome *P*-450-mediated metabolism of the individual enantiomers of the antidepressant agent reboxetine in human liver microsomes. *Drug Metab Dispos* 1999;27:1334–1340. [PubMed: 10534319]
12. Dischino D, Welch M, Kilbourn M, Raichle M. Relationship between lipophilicity and brain extraction of C-11-labeled radiopharmaceuticals. *J Nucl Med* 1983;24:1030–1083. [PubMed: 6605416]
13. Loscher W, Potschka H. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog Neurobiol* 2005;76:22–76. [PubMed: 16011870]
14. Andree B, Seneca N, Schou M, Mozley PD, Potter W, Farde L, Gulyas B, Halldin C. Demonstration of selective norepinephrine transporter binding in human brain using PET and [C-11]MeNER. *Eur J Nucl Med Mol Imaging* 2004;31:S208.
15. Haradahira T, Zhang MR, Maeda J, Okauchi T, Kida T, Kawabe K, Sasaki S, Suhara T, Suzuki K. A prodrug of NMDA/glycine site antagonist, L-703,717, with improved BBB permeability: 4-acetoxy derivative and its positron-emitter labeled analog. *Chem Pharm Bull (Tokyo)* 2001;49:147–150. [PubMed: 11217099]
16. Smith HR, Beveridge TJ, Porrino LJ. Distribution of norepinephrine transporters in the non-human primate brain. *Neuroscience* 2006;138:703–714. [PubMed: 16427744]
17. Donnan G, Kaczmarczyk S, Paxinos G, Chilco P, Kalnins R, Woodhouse D, Mendelsohn F. Distribution of catecholaminergic uptake sites in human brain as determined by quantitative [3H] mazindol autoradiography. *J Comp Neurol* 1991;304:419–434. [PubMed: 2022757]
18. Tejani-Butt S. [3H]Nisoxetine: A radioligand for quantitation of norepinephrine uptake sites by autoradiography or by homogenate binding. *J Pharm Exp Ther* 1992;260:427–436.
19. Zoghbi SS, Shetty HU, Ichise M, Fujita M, Imaizumi M, Liow JS, Shah J, Musachio JL, Pike VW, Innis RB. PET imaging of the dopamine transporter with ¹⁸F-FECNT: a polar radiometabolite confounds brain radioligand measurements. *J Nucl Med* 2006;47:520–527. [PubMed: 16513622]

Fig. 1.
Time-course for the metabolism of (*S,S*)-[¹¹C]MeNER measured in human and monkey plasma.

Fig. 2. Rat regional brain distribution of (S,S)-[^{11}C]MeNER and its radiometabolite. The uptake and the regional brain distribution of [^{11}C]MeNER and its radiometabolite at 30 min after intravenous injection of 1.73 ± 0.3 mCi; 6.87 ± 1.03 nmol/kg.

Fig. 3. Radioactivity concentrations in two brain regions and in arterial plasma (average of six baseline experiments) after injection of (S,S)-[^{11}C]MeNER into healthy human volunteers.

Scheme 1.

Putative metabolism scheme based on the metabolism of reboxetine ($R = \text{Et}$), which is an *O*-ethyl homolog of (*S,S*)-MeNER ($R = \text{Me}$) [6].