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5-HTT and 5-HT$_{1A}$ receptor occupancy of the novel substance vortioxetine (Lu AA21004). A PET study in control subjects

Per Stenkrona, Christer Halldin, Johan Lundberg*

Department of Clinical Neuroscience, Karolinska Institutet, Karolinska University Hospital Solna, Building R5, 171 76 Stockholm, Sweden

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Abstract
Vortioxetine (Lu AA21004) is a new potential substance for the treatment of anxiety and mood disorders. It has high affinity for the 5-HT transporter (5-HTT) and moderate affinity for the 5-HT1A receptor in vitro. Positron emission tomography (PET) has commonly been used to examine the relation between dose/plasma concentration and occupancy to predict relevant dose intervals in a clinical setting. In this study 11 control subjects were examined with PET and [^{11}C]MADAM at baseline, after a single dose and after 9 days of dosing with Lu AA21004 (2.5, 10 or 60 mg) for quantification of 5-HTT occupancy. Four subjects were examined with PET and [^{11}C]WAY 100635 at baseline, after a single dose and after 9 days of dosing of Lu AA21004 (30 mg) for quantification of 5-HT$_{1A}$ occupancy. To allow for quantification of binding in the raphe nuclei, PET data were analyzed using wavelet aided parametric imaging. 5-HTT occupancy ranged from 2 (mean, 2.5 mg day 1) to 97% (60 mg day 9). The apparent affinity of Lu AA21004 binding to 5-HTT (K_D^{ND}) was calculated to 16.7 nM (R=0.95), and the corresponding oral dose (K_D^{ND}-*dose*) to 8.5 mg (R=0.91). No significant occupancy of 5-HT$_{1A}$ receptors was found after dosing of 30 mg Lu AA21004. Based on the literature and the present [^{11}C]MADAM binding data, a dose of 20–30 mg Lu AA21004 is suggested to give clinically relevant occupancy of the 5-HTT.

1. Introduction

Selective serotonin (5-HT) reuptake inhibitors (SSRIs) are well established for the treatment of anxiety and mood disorders (Vaswani et al., 2003). The treatment is associated with a modest response rate of about 60% and an average time to onset of antidepressant effect of about 3 weeks (Barbui and Hotopf, 2001; Kasper et al., 2006). This makes research for more optimal treatment alternatives warranted. The addition of the 5-HT$_{1A}$ receptor partial agonist pindolol to SSRI treatment has been associated with a more rapid antidepressant effect, both in animal models and in patients suffering from major depressive disorder (Artigas et al., 1996; Ballesteros and Callado, 2004). Also, the addition of 5-HT1A agonism to SSRI

*Corresponding author. Tel.: +46 851773720; fax: +46 51771753.
E-mail address: johan.lundberg@ki.se (J. Lundberg).

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has been suggested to rapidly desensitise the 5-HT$_{1A}$ receptor and thus theoretically shorten the time to onset of clinical effects (Bang-Andersen et al., 2011).

The novel compound vortioxetine (Lu AA21004; 1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine-hydrobromide) has high affinity for the human 5-HT transporter in vitro (5-HTT; Ki = 1.6 nM) and medium affinity, and agonistic properties, for the human 5-HT1A receptor (Ki = 15 nM). It also has affinity for the human 5-HT$_{3A}$ (Ki = 3.7 nM), 5-HT$_{1B}$ (Ki = 33 nM) and 5-HT$_7$ receptor (Ki = 19 nM) in vitro (Bang-Andersen et al., 2011). The compound is thought to combine 5-HT reuptake inhibition with agonism of the 5-HT$_{1A}$ receptor. The compound is the subject of 31 clinical trials for the use in Major Depressive Disorder and Generalized Anxiety Disorder (http://clinicaltrials.gov as of April 24, 2012).

Positron emission tomography (PET) is increasingly applied to confirm drug binding to targets in the living human brain and to elucidate relationships between the degree of binding (occupancy) at the target protein and concentrations in extra cerebral compartments more readily available for sampling, e.g. plasma (Farde, 1996). The application of PET in drug development has thus the potential to predict therapeutic dose ranges from relatively small numbers of healthy volunteers. Previous PET studies of clinical samples treated with serotonergic antidepressants suggest doses corresponding to 65–80% 5-HTT occupancy to be clinically relevant (Lundberg et al., 2012; Meyer et al., 2004).

The raphe nuclei is a key region of interest in drug treatment of major depressive disorder for three reasons: (1) many, but not all, case control PET studies have shown lower binding potential (BP_{ND}) of both 5-HTT and 5-HT$_{1A}$ receptors in the raphe nuclei in cases compared to controls (Meyer, 2007; Savitz et al., 2009), (2) the acute effect of SSRI treatment is increased 5-HT concentration in the raphe nuclei but not other regions (Adell and Artigas, 1991; Bel and Artigas, 1992), and (3) the suggested mechanism of action of pindolol is a blockade of inhibitory autoreceptors on 5-HT neuron soma and dendrites in the raphe nuclei (Bel and Artigas, 1992). Also the benefit from 5-HT$_{1A}$ agonists is thought to be driven by occupancy of 5-HT$_{1A}$ receptors in the raphe nuclei (Bang-Andersen et al., 2011).

[^{11}C]MADAM and [^{11}C]WAY 100635 are two suitable PET radioligands for regional quantification of 5-HTT and 5-HT$_{1A}$ receptor binding respectively (Farde et al., 1998; Gunn et al., 2000; Lundberg et al., 2005). Both ligands have been used previously for occupancy studies in human subjects (Andrée et al., 2000; Lundberg et al., 2007).

The aim of the present study was to examine the relation between plasma concentration of Lu AA21004 and occupancy at 5-HTT and 5-HT$_{1A}$ receptors in the raphe nuclei. The primary hypothesis was that Lu AA21004 occupies the 5-HTT in a dose dependent fashion. The secondary hypothesis was that Lu AA21004 also occupies the 5-HT$_{1A}$ receptor.

2. Experimental procedures

2.1. Subjects and design

The study was approved by the Regional Ethical Review Board in Stockholm and the Radiation Safety committee of the Karolinska University Hospital. Seventeen male subjects (age 20–45 years, BMI 19–29 kg/m^2) were randomised after giving verbal and written informed consent. All subjects were healthy based on medical history, psychiatric interview, physical examination, electrocardiogram (ECG), blood and urine analysis, and magnetic resonance imaging (MRI) of the brain. They were not treated with any medication and they were all non-smokers. The study had a multiple dose, open label design. The occupancy of the 5-HT transporter by Lu AA21004 was investigated at 3 dose levels (2.5 mg/day, 10 mg/day and 60 mg/day) in 3 groups of 4 subjects. The occupancy of the 5HT$_{1A}$ receptor was investigated in one group of 4 subjects at one dose level (30 mg/day). Sixteen subjects were dosed for 9 consecutive days and 15 subjects participated in all 3 PET measurements. PET measurements were performed at baseline (from 2 weeks prior to dosing), following the first dose (Day 1) and following the final dose (Day 9). The post-treatment PET experiments were performed at expected T_{max}, 7 h post-dose. All PET measurements were performed at a similar time of day. During the multiple dose periods the subjects were confined to the Quintiles AB Unit in Uppsala. At selected time intervals blood samples were obtained for pharmacokinetic analyses of Lu AA21004. Tolerability and safety were assessed throughout the study by monitoring adverse events and by clinical laboratory safety tests, ECG, weight, vital signs assessments and physical examinations.

2.2. MRI

MRI scans were performed on a 1.5 T unit (General Electric, Signa). Two examinations were made in one session during 15 min. The first was T2-weighted for clinical evaluation regarding pathology. The second was T1-weighted for co-registration with PET and delineation of anatomical brain regions or regions of interests (ROI's). The T2 sequence was a 2-D fast spin echo protocol with the following settings: TR 5000 ms, TE 68, FOV 260 × 260 mm^2, 44 × 3.0 mm^2 slices, slice gap 0.125 mm, matrix 256 × 256, 1 NEX. The T1 sequence was a 3-D SPGR protocol with the following settings: TR 21 ms, TE 6 ms, flip angle 35°, FOV 260 mm, matrix 256 × 256 × 156, 156 × 1.0 mm^2 slices, 1 NEX. The T1 sequence was optimized for a minimum of scanning time and a maximum of spatial resolution and contrast between grey and white matter.

2.3. Radiochemistry

[^{11}C]MADAM was obtained by methylation of ADAM using [^{11}C]methyl iodide, as described previously (Hall et al., 1997; Tarkiainen et al., 2001). Between 288 and 316 MBq was injected intravenously (N = 33). The specific radioactivity of the radioligand injected varied between 3887 and 110,701 Ci/mmol, corresponding to a mass injected of 0.02–0.6 μg (N = 29, analysis failed for technical reasons on four occasions).

[^{11}C]WAY-100635 was prepared from ^{11}C-acylation of WAY100634 with carbonyl-^{11}C-cyclohexanecarbonyl chloride as described previously (Hall et al., 1997). Between 153 and 320 MBq was injected intravenously (N = 12). The specific radioactivity of the radioligand injected varied between 310 and 2246 Ci/mmol, corresponding to a mass injected of 1.5–11.4 μg (N = 11, analysis failed due to lack of product at one occasion).

2.4. PET experimental procedure

PET measurements were performed on an ECAT Exact HR 47 system (CTI/Siemens, Knoxville, TN, USA) run in three-dimensional mode with dual-energy Windows scatter correction (Wienhard et al., 1994). A three-ring detector block architecture gives a 15 cm wide field of view (FOV). The transaxial resolution of the reconstructed images is 3.8 mm full width at half maximum (FWHM) at the centre of the FOV, 4.5 mm FWHM tangentially, and 7.4 mm radially at 20 cm from the centre. The axial resolution is 4 mm FWHM at the

centre and 6.8 mm at 20 cm from the centre. Prior to each emission a transmission scan of 10 min was performed using three rotating 68Ge–68Ga sources. After correction for attenuation, random and scattered events, images were reconstructed using a Hann filter (2 mm FWHM). The reconstructed volume was displayed as 47 horizontal sections with a centre-to-centre distance of 3.125 mm and a pixel size of 2×2 mm^2.

In each PET measurement, the subject was placed recumbent with his head in the PET system. A head fixation system with an individual plaster helmet was used to minimize movement (Bergstrom et al., 1981). A sterile physiological phosphate buffer (pH=7.4) solution containing the radioligand was injected as a bolus during about10 s into a cannula inserted into the right antecubital vein. The cannula was then immediately flushed with 10 ml saline.

Brain radioactivity was measured in a series of consecutive time frames. After injection with [^{11}C]MADAM, the examination lasted for 93 min and consisted of 20 frames ($3 \times 1'$; $4 \times 3'$; $13 \times 6'$). After injection with [^{11}C]WAY100635 the examination lasted for 69 min and consisted of 16 frames ($3 \times 1'$; $4 \times 3'$; $9 \times 6'$).

2.5. Coregistration

A co-registration procedure was applied to align the PET and MRI datasets. For each subject the MR image was spatially normalized to position the anterior–posterior (AC–PC) commissural line in the horizontal plane, and the inter-hemispheric plane orthogonal to the AC–PC plane. The MR normalized image was resampled and cropped to generate a $256 \times 256 \times 141$ mm^3 matrix with 1 mm^2 pixels before it was used for manual definitions of regions of interest (ROIs). The resampled MR images were co-registered to the corresponding PET images. Co-registration was done using the normalized mutual information method implemented in SPM5 (Maes et al., 1997).

2.6. Regions of interest

The raphe nuclei were not visible on the MR images. Instead the ROIs of the raphe nuclei were defined by a standardised rectangle of the brainstem in all sagittal planes including the aqueductus cerebri at the level of pons (0.49 ± 0.10 ml, (mean $\pm$ SD)). ROIs were defined for the raphe nuclei and cerebellum. Cerebellar ROIs were defined manually using the Human Brain Atlas software (Roland et al., 1994) according to anatomical boundaries (excluding vermis) and delineated in 5 consecutive sections on the horizontal MR images and transferred to the corresponding reconstructed PET images. In a secondary analysis of [^{11}C]WAY 100635 binding in post synaptic regions, ROIs were defined in the same manner for the anterior cingulate cortex (sagittal plane), dorsolateral prefrontal cortex (coronal plane), dorsomedial prefrontal cortex (coronal plane), occipital cortex and temporal cortex (sagittal plane). For cortical regions the delineation was over inclusive, including also surrounding CSF and white matter. The SPM5 software was used to segment the normalized MRI images into grey and white matter and CSF. The grey matter masks were applied to the manually delineated cortical ROIs in order to include only pixels belonging to grey matter.

2.7. Quantification of [^{11}C]MADAM and [^{11}C] WAY-100635 binding

Parametric images were generated using the stationary wavelet transform-based parametric mapping framework (S-WAPI) implemented in Matlab R2007b for Windows, as described in detail elsewhere (Cselenyi et al., 2006). In brief, the original PET images were transformed to the wavelet space. The depth of the decomposition was 2, the length of the filter kernels was 22. The coefficients of the resulting dynamic wavelet transform were analyzed quantitatively with Logan's graphical estimation using a multi-linear regression to fit the linear part of the curve (Turkheimer et al., 2003). Hard thresholding was performed on the wavelet coefficients via comparing their area under the curve (AUC) value to that of the reference region. The end product of the calculations was a parametric wavelet transform describing the distribution of the distribution volume ratio (DVR). Then, a wavelet reconstruction was applied to the parametric transform to yield the three-dimensional (3D) parametric map of DVR in normal space. The DVR map was transformed to a BP_{ND} map by subtracting one from the DVR values.

The use of parametric images was motivated by our intention to calculate occupancy in the raphe nuclei, a small region with noisy time activity curves where standard reference tissue models have less advantageous reproducibility (Lundberg et al., 2006; Parsey et al., 2000). Data were generated using S-WAPI since the application of wavelet filters has been shown to provide more reliable estimates of radioligand binding (Cselenyi et al., 2006).

The ROIs were superimposed on the parametric PET images using the coregistration parameters to obtain the BP_{ND} values for each region. 5-HTT drug occupancy (ΔBP_{ND} (%)) was calculated according to the following equation:

$$\Delta BP_{ND} = \left(1 - \frac{BP_{ND(treatment)}}{BP_{ND(baseline)}}\right) \times 100 \quad (1)$$

where BP_{ND} (baseline) refers to the BP_{ND} calculated at the baseline condition and BP_{ND} (treatment) to the BP_{ND} calculated after either one or nine doses of Lu AA21004.

2.8. Determination of plasma concentrations

The blood samples were inverted 6–8 times upon collection and transferred to a pre-cooled centrifuge within 5 min of collection. Samples were centrifuged within 15 min of collection, after which they were stored at approximately -20 °C. Samples were shipped on dry ice to Aptuit Limited, Edinburgh, United Kingdom, for analysis. Determination of Lu AA21004 in plasma was done using solid phase extraction followed by HPLC with tandem mass spectrometric detection, with the limit of quantification (LLOQ) of 0.4 ng/ml. For subject 124 and 102 the plasma analysis failed to reach quality acceptance criteria and the results were thus excluded (Table 1).

2.9. Calculation of K_D^{ND}

The relationship between occupancy and drug plasma concentration can be described by a curvilinear function given by the following equation as derived from the law of mass action:

$$B = \frac{B_{max} \times F}{K_D^{ND} + F} \quad (2)$$

where B is the concentration of ligand bound to receptor, B_{max} the number of available receptors, F the concentration of unbound ligand and K_D^{ND}, the apparent inhibition constant (Karlsson et al., 1995). The affinity is called apparent when plasma concentration is used as an estimate for free fraction in plasma (f_p), and when the concentration of endogenous ligand (5-HT) and non-specific binding in brain (f_{ND}) is not corrected for.

In drug occupancy studies, Eq. (2) may be rewritten as follows:

$$\text{Occupancy} = \frac{occ_{max} \times C_s}{K_D^{ND} + C_s} \quad (3)$$

where occ_{max} is the maximal occupancy induced by the drug and C_s is the plasma concentration of the drug. This is done under the assumption that there is a linear relationship between drug concentration in brain and plasma so that F may be substituted with C_s and that occ_{max} is 100% and that the nondisplaceable distribution volume of Lu AA21004 is uniform. A population based K_D^{ND} was calculated by relating all the calculated 5-HTT occupancy

values and all mean plasma concentrations of Lu AA21004 at 7–8.5 h post dose, i.e., during the PET measurement, by means of a least squares minimization procedure. Similarly, assuming linear relation between oral dose and plasma concentration of Lu AA21004, the K_D^{ND} for oral doses was calculated.

2.10. Statistics

Due to the small sample size, no assumption about normal distribution was made. Thus descriptive statistics include median values and all tests were non-parametric. For between groups comparisons the Kruskal–Wallis Test was used. For paired comparisons the Wilcoxon Signed Ranks Test was used. The significance level was set to 0.05. Tests were performed using PASW Statistics 20 (SPSS, IBM Corporation, Somers, NY, USA).

3. Results

Seventeen subjects were randomized. One subject was excluded before dosing due to technical problems. No serious adverse event was reported in any of the 16 subjects dosed. One subject treated with 60 mg Lu AA21004 daily was withdrawn after vomiting, the only severe treatment-emergent adverse event (TEAE) reported in the study. Thirty-nine TEAEs were reported by 11 of the 16 subjects dosed during this study. The most common TEAEs by subject incidence were headache (25%), nausea (25%), vomiting (13%), flatulence (13%) and pruritus (13%). Moderate adverse events included eye pain, tremor, nausea, diarrhoea, abdominal pain and vomiting. No TEAE was reported in the 2.5 mg group. There were no clinically significant changes in laboratory safety test results, vital signs, weight or 12-lead ECG data following administration of Lu AA21004.

The plasma concentrations of Lu AA21004 reached t_{max} at 6 (10 and 30 mg) to 14 (2.5 and 60 mg) hours post dose (median) on day 1 and 7.5 to 10 h post dose on day 9 (Figure 1). The PET measurements were started 7 h post dose and the plasma concentrations were thus either at or around C_{max}. The plasma concentrations at time of PET (ie 7–8.5 h post dose) were significantly different with regards to dose on both day one and day nine (Independent Samples Kruskal Wallis test; $p=0.01$; Tables 1 and 2).

Inspection of parametric PET images after injection of [^{11}C]MADAM showed lower BP_{ND} in the raphe nuclei at posttreatment conditions compared to baseline suggesting quantifiable occupancy of Lu AA21004 (Figure 2). The median 5-HTT occupancy for Lu AA21004 2.5 mg daily was 1% (at day one) and 25% (at day one and nine, respectively), for 10 mg daily 38% and 53%, and for 60 mg daily 79% and 98%. It could be shown that the occupancy on both day one and nine increased with dose (Independent Samples Kruskal Wallis Test; $p<0.05$; Table 1).

Fitting occupancy data from 18 PET measurements in 9 subjects at three different doses of Lu AA21004 to the corresponding plasma concentration data, the K_D^{ND} based on day 1 and day 9 data separately were calculated to 13.6 ($R=0.95$) and 20.0 ($R=0.96$) nM respectively. Similarly, by pooling day 1 and day 9 the K_D^{ND} was calculated to be 16.7 nM ($R=0.95$; Figure 3). When fitting occupancy data for day nine, when steady state was expected, to the corresponding oral doses (2.5, 10 and 60 mg) the oral dose needed to occupy 50% of the 5-HTT (K_D^{ND}-oral dose) was calculated to be 8.5 mg ($R=0.91$).

Four subjects were examined with PET and the radioligand [^{11}C]WAY-100635 before and after dosing of Lu AA21004. The plasma concentrations of Lu AA21004 at time of PET were shown to differ between day 1 and day 9 (median values 30.2 nM vs 105 nM, $p<0.05$ Independent Samples Kruskal–Wallis Test).

The difference in [^{11}C]WAY-100635 BP_{ND} between baseline and after treatment with LU AA21004 was not statistically significant (Table 2; BP_{ND} baseline vs day 9; Related Samples Wilcoxon Signed Ranks Test, $p=0.27$). An extended analysis of BP_{ND} also in the anterior cingulate gyrus, dorsolateral prefrontal cortex, dorsomedial prefrontal cortex and occipital cortex was done. For all these regions the average variability in BP_{ND} values was lower than the known test-retest variability (data not shown). There was a strong correlation between the calculated occupancy in the Raphe nuclei and that of an average of all the regions examined (Kendall's Tau: 1.00, $p<0.01$). Finally, as indicated from Figure 4, there was no hyperbolic relation between LU AA21004 plasma concentration and the calculated occupancy value in the Raphe nuclei or in an average of all the examined regions.

4. Discussion

Vortioxetine (Lu AA21004) is a novel compound currently in clinical trials for MDD and GAD. In the present study we examined the relation between plasma concentration of Lu AA21004 and occupancy of the 5-HTT and 5-HT$_{1A}$ receptor in a group of control subjects after single and multiple doses.

Adverse events, clinical safety laboratory tests, vital signs, weight, ECG and physical examinations were recorded throughout the study period. The number of subjects reporting any Treatment Emergent Adverse Event (TEAE) ranged from none (2.5 mg) to all (60 mg) with one serious TEAE (vomiting) for 60 mg of Lu AA21004. This indicates that the dose range was relevant for identifying pharmacodynamic effects.

The time taken to reach steady-state was assessed by visually inspecting the individual plasma Lu AA21004 predose concentration–time profiles up to the last day of dosing (Figure 1). Plasma concentrations of Lu AA21004 tended to approach or reach steady-state following 9 days once-daily dosing. Thus, the occupancy values calculated based on day one data is what should be expected after a single oral dose of Lu AA21004 and the occupancy values based on day nine data reflects continuous treatment.

The range of 5-HTT occupancy calculated from [^{11}C]MADAM BP_{ND}'s after single and multiple dosing of 2.5–60 mg of Lu AA21004 was −9 to 99% (Table 1). The high occupancy values of 99% confirms saturability of [^{11}C]MADAM binding to 5-HTT and thus its usefulness for quantifying Lu AA21004 5-HTT binding in vivo (Halldin et al., 2001). The wide range of occupancy values was beneficial when fitting the binding hyperbolic function (Figure 3). The apparent inhibition constant (K_D^{ND}) was 16.5 nM. As a comparison, K_D^{ND} based on day 1 and day 9 data only were calculated to 13.6 ($R=0.95$) and 20.0 ($R=0.96$) nM respectively. The low occupancy values and low plasma concentrations on day 1, especially for the

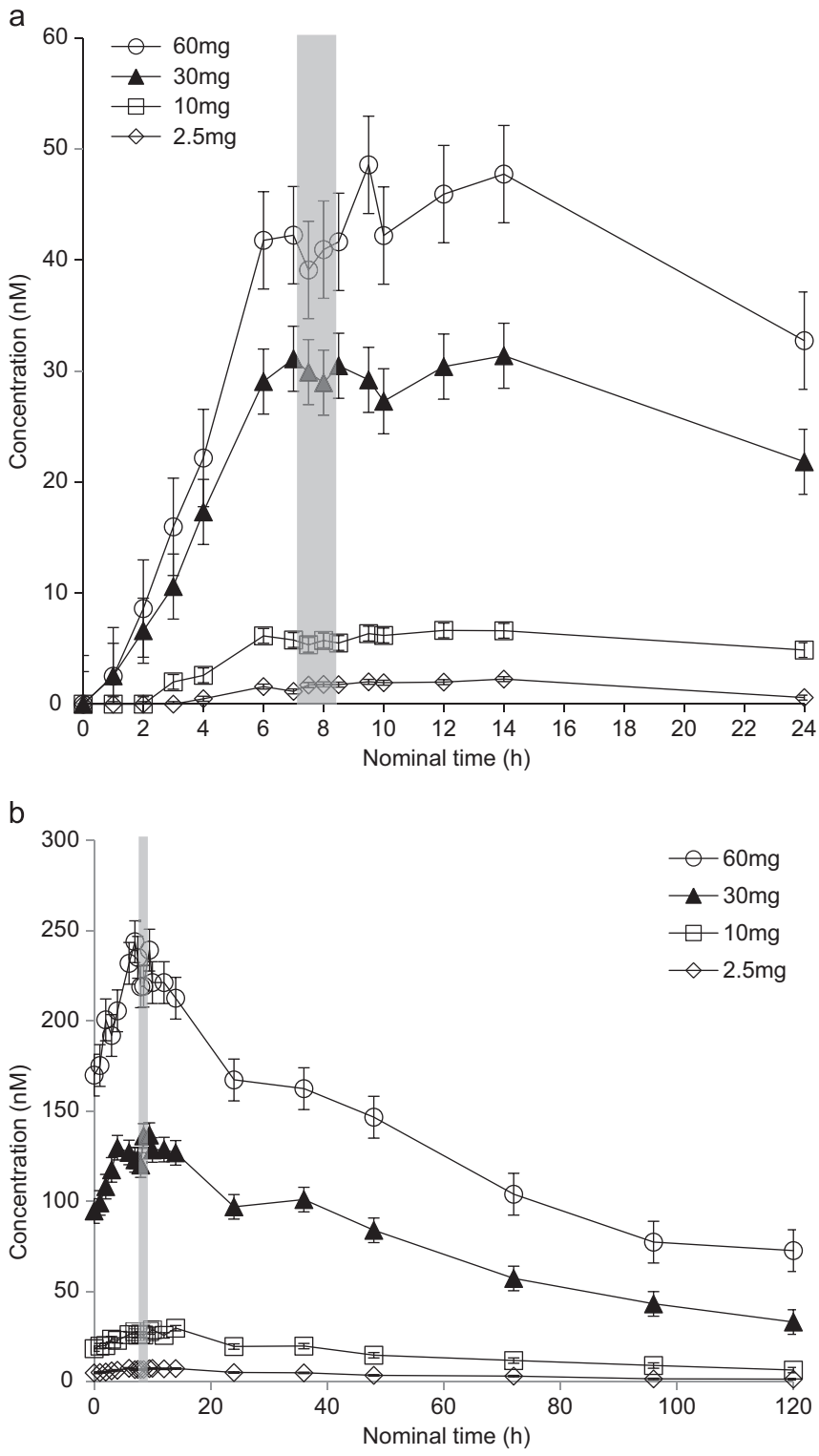

Figure 1 (A) Mean (±SE) plasma concentrations for Lu AA21004 following single oral administration of Lu AA21004 at 2.5, 10, 30 and 60 mg: Day 1. The grey bar indicates the time of PET experiments (7–8.5 h post dose), (B) mean (±SE) plasma concentrations for Lu AA21004 following repeated oral administration of Lu AA21004 at 2.5, 10, 30 and 60 mg: Day 9. The grey bar indicates the time of PET experiments (7–8.5 h post dose).

subjects dosed with 2.5 mg Lu AA21004, decrease the reliability of the calculated inhibition constant. Thus, the K_D^{ND} based on day 9 measurements should be more valid.

Studies in clinical samples treated with 5-HTT occupying substances have reported 80% occupancy at recommended clinical doses (Meyer et al., 2004; Suhara et al., 2003). In a

Table 1 Pharmacokinetic and [^{11}C]MADAM pharmacodynamic data by patient and dose.

Subject	Dose (mg/day)	Plasmaconc (nM)[a]		5-HTT BP_{ND} [b]			5-HTT occupancy (%)[b]	
		Day1	Day9	Baseline	Day1	Day9	Day1	Day9
121	2.5	1.54	5.67	0.98	1.07	0.92	−9	6
123	2.5	2.08	10.3	1.53	1.48	1.19	3	22
124	2.5	nr	nr	1.36	1.17	0.85	14	37
222	2.5	1.56	4.22	1.04	1.03	0.75	1	28
101	10	21.0	37.6	0.74	0.39	0.22	47	70
102	10	nr	nr	0.94	0.89	0.68	5	27
103	10	5.97	16.5	0.94	0.54	0.51	43	46
104	10	5.21	25.1	0.86	0.53	0.35	38	60
105	60	47.0	337	1.41	0.38	0.08	73	94
106	60	36.8	200	1.06	0.22	0.01	79	99
108	60	39.2	151	1.03	0.21	0.02	80	98

<LLOQ: Below lower limit of quantification.
[a]At time of PET. Average value based on four samples.
[b]In the Raphe nuclei. nr=no result; sample failed to meet acceptance criteria following reprocessing.

Table 2 Pharmacokinetic and [^{11}C]WAY 100635 pharmacodynamic data for the Raphe nuclei by patient and dose.

Subject	Plasmaconc (nm)		5-htt BP_{ND} (WS CV)			5-htt occupancy (%)	
	Day1	Day9	Baseline	Day1	Day9	Day1	Day9
131	27.3	194	0.86	1.07(15)	1.60(42)[a]	−24	−85
132	33.0	107	0.84	0.76(7)	0.51(34)[a]	10	39
133	25.7	98.4	0.51	0.48(4)	0.99(46)[a]	5	−96
134	33.3	103	1.54	1.33(10)	1.79(11)	13	−17

WS CV=The within subject coefficient of variance (within subject standard deviation/mean value (%)).
[a]WS CV exceeds the WS CV previously reported for test-retest data in the same region (30%, (Parsey et al., 2000)). Pharmacodynamic data are from the Raphe nuclei.

recent study of seven antidepressant substances in 20 patients in remission from major depression the mean 5-HTT occupancy was 67% (95% CI: 61–74; (Lundberg et al., 2012)). Importantly, the lower limit of clinically relevant 5-HTT occupancy has not yet been identified. For other serotonergic targets similar information on clinically relevant occupancy values has not yet been established. In the present study, assuming a linear relation between oral dose and plasma concentration at steady state, the dose required for 50% occupancy was calculated to 8.5 mg (K_D^{ND}-oral). Similarly 70% requires 20 mg and 80% requires 37 mg. The 5-HTT occupancy values in the present study can be guidance for upcoming clinical trials with Lu AA21004 where daily doses of in the range of 20–30 mg are recommended, in order to reach a 5-HTT occupancy of about 70-80%. However, this study is not designed to investigate the correlation between oral dose and plasma concentration of Lu AA21004. Such data from a larger sample can with the current K_D^{ND}-oral be of additional guidance for oral dose recommendation.

5-HTT occupancy and affinity calculations were based on BP_{ND} measurements in the raphe nuclei. This was motivated by the key role of the serotonergic neurons of this region in the pathophysiology and pharmacology of MDD. Although the baseline BP_{ND} of the raphe is high the region is relatively small and difficult to delineate. Therefore to improve reliability operationally defined ROI's for the raphe and wavelet parametric images were applied. In previous studies of 5-HTT occupancy, the putamen has commonly been used as an index region due to good test-retest characteristics (Lundberg et al., 2012; Meyer et al., 2004). In the present study a comparison of these results with those of a secondary analysis of occupancy in the putamen showed no significant difference (Wilcoxon Signed Rank Test, $p=0.159$). On the contrary, there was a good correlation between the two regions (Kendall's Tau: 0.81, $p<0.001$; Figure 3). This crossvalidates the use of the Raphe nuclei as the primary region of analysis of 5-HTT data in this study.

The data for the 5-HT$_{1A}$ receptor are limited as only four subjects were examined with PET and [^{11}C]WAY-100635. In the raphe nuclei, the primary region of analysis, or in any of the six regions in the secondary analysis, 30 mg of Lu AA21004 was not shown to occupy the 5-HT$_{1A}$ receptor although the absolute variability in BP_{ND} values between baseline and day 9 exceeded the known test–retest variability in three of the four subjects (Table 2 (Parsey et al.,

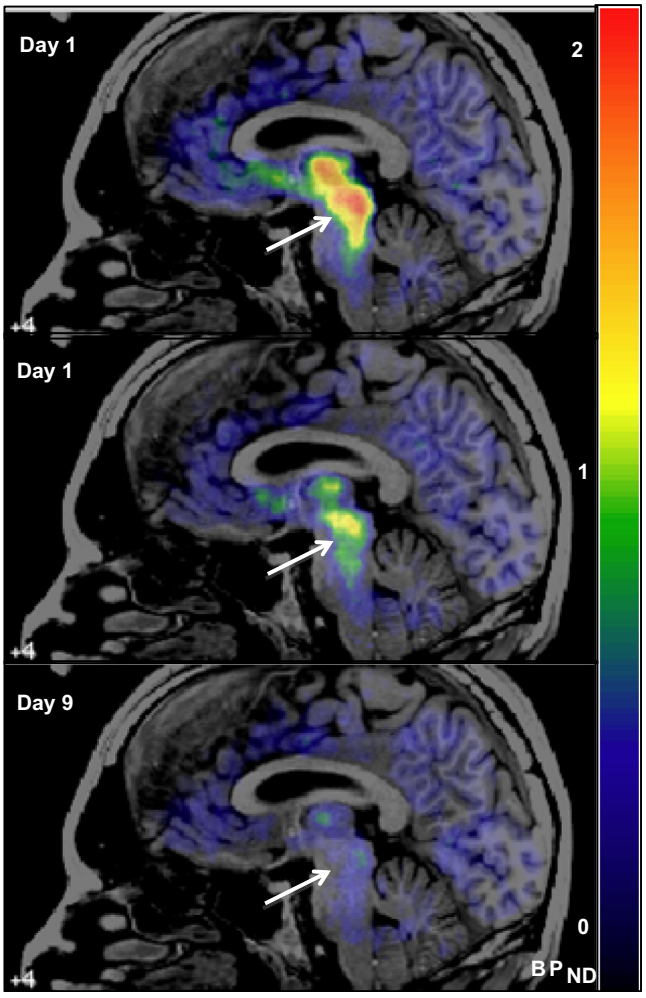

Figure 2 Parametric sagittal images of central serotonin transporter binding potential at baseline, day 1 and day 9 after 10 mg Lu AA 21004 qd. The arrows are pointing at the Raphe nuclei.

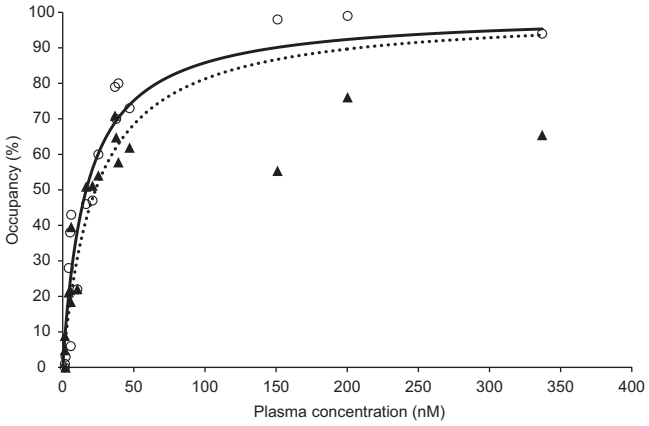

Figure 3 Illustration of the hyperbolic relation between plasma concentration of Lu AA21004 and 5-HTT occupancy. Open circles and solid line represents Raphe data. Closed triangles and dashed line represents Putamen data.

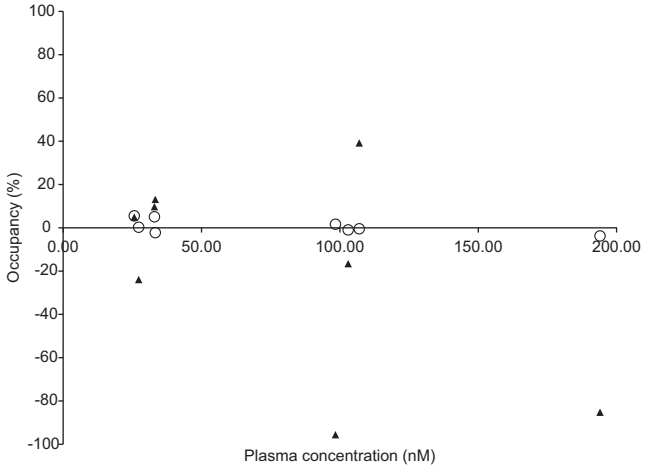

Figure 4 Scatter plot illustrating the lack of a hyperbolic relation between plasma concentration of Lu AA21004 and 5-HT_{1A}-receptor occupancy. Open circles represent Raphe data. Closed represents an average of data from all examined regions (anterior cingulate cortex, dorsolateral prefrontal cortex, dorsomedial prefrontal cortex, occipital cortex and temporal cortex).

2000)). This was not the case in any of the other regions examined in the extended analysis. As a consequence, calculation of occupancy values in these regions can be questioned. Still, there was a correlation between an average occupancy value for all regions and that of the Raphe nuclei (Kendall's Tau: 1.00, $p<0.01$), validating the use of the Raphe nuclei as the primary region of analysis also of 5-HT_{1A}-receptor data in this study.

According to in vitro data the affinity to the 5-HT_{1A} receptor is 4–20 times lower than to the 5-HTT. It is thus not excluded that a higher dose may be associated with measurable occupancy also of the 5-HT_{1A} receptor. 5-HT_{1A}-receptors occur in high and low affinity (HA/LA) states. The HA state has been suggested to be the functionally relevant state. Lu AA21004 has agonistic properties, and should preferentially bind to HA (Clawges et al., 1997). [^{11}C]WAY-100635 binds to the HA and LA conformations with similar affinity. Thus, in the present study there is a reduced signal-to-noise ratio of Lu AA21004 binding to the HA state receptor as compared to that using an agonist radioligand such as [^{11}C]CUMI-101. The reduced signal to noise may have obscured a low Lu AA21004 occupancy of 5-HT_{1A}-receptors in the HA state. To quantify the relative Lu AA21004 occupancy of the HA/LA receptor states a study protocol where a 5-HT_{1A} receptor antagonist such as [^{11}C]WAY-100635 is combined with an agonist such as [^{11}C]CUMI-101 should be used (Kumar and Mann, 2007).

This study do not address the potential occupancy of other serotonergic receptors for which Lu AA21004 has affinity in vitro in the nanomolar range (5-HT_{3A}, 5-HT_{1B}, and 5-HT_7). It remains to be shown if and to what extent this is of importance for the clinical effect of Lu AA21004.

The sample size (n=11) was sufficient to verify the primary hypothesis, that Lu AA21004 occupies the 5-HTT in the raphe nuclei in a dose dependent fashion. The sample size (n=4) was however too small to either verify or reject the secondary hypothesis, that Lu AA21004 also occupies the 5-HT_{1A} receptor.

The study was performed in control subjects without ongoing psychiatric disease. The 5-HTT and 5-HT_{1A} receptor BP_{ND} has repeatedly been shown to be lower in several brain regions of patients with major depression when compared to control subjects. It has not been shown if this is due to a decrease in B_{max} or increase in K_D. A change in K_D may produce differences in affinity between patients and controls. Thus, a verification of the finding in patients is warranted. A replication of calculated K_D data from this study should support the notion that MDD is associated with a decrease in B_{max} rather than an increase in K_D. Also, a replication should benefit the clinical dose recommendations of Lu AA21004.

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Contributors

Per Stenkrona (PS), Christer Halldin (CH) and Johan Lundberg (JL) designed the study and wrote the protocol together with H. Lundbeck A/S. PS, CH and JL managed the literature searches and analyses. JL wrote the first draft of the manuscript. All authors have contributed to and approved the final manuscript.

Conflict of interest

None.

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