BCPT Basic & Clinical Pharmacology & Toxicology

Basic & Clinical Pharmacology & Toxicology, 2012, **110**, 401–404 Doi: 10.1111/j.1742-7843.2011.00810.x

Short Communication

Occupancy of the Serotonin Transporter after Administration of Lu AA21004 and its Relation to Plasma Concentration in Healthy Subjects

Johan Areberg, Michael Luntang-Jensen, Birgitte Søgaard and Dorrit Ø. Nilausen

Division of Clinical and Quantitative Pharmacology, H. Lundbeck A/S, Valby, Denmark

(Received 5 July 2011; Accepted 20 September 2011)

Owing to the lack of biologically based clinical measures and reliable biomarkers for many indications within the CNS area, the use of imaging tools, such as ligand-based positron emission tomography (PET), has become more and more important in the development of new CNS drugs. A well-grounded and reliable relationship between the plasma concentration of the drug and the occupancy of the receptor/transporter is a powerful tool to investigate the mechanism of action of a new drug and to guide dose selection in patients.

Imaging of the serotonin transporter (5-HTT) by means of either PET or single photon emission computed tomography (SPECT) is one of the most useful methods in the neuro- and psychopharmacological field. A number of different ligands have been tested and used over the past 20 years. The first radiotracer ligand to be used in human beings was the SPECT ligand [^{123}I]2-β-carbomethoxy-3-β-(4-iodophenyl)-tropane (β-CIT), and in 1995, the first successful PET imaging study of the distribution of the 5-HTT in the brain of human volunteers using [^{11}C](+)McN5652 (trans-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl]-pyrrolo-[2,1-a]-isoquioline) was reported [1]. In 2005, the first study with the PET ligand [^{11}C]-MADAM (*N*,*N*-dimethyl-2-(2-amino-4-methylphenylthio) benzylamine) in human beings was reported [2], and the suitability of the cerebellum as a reference region for non-specific [^{11}C]-MADAM binding could be confirmed, thus paving the way for experimentally less demanding approaches, such as the simplified reference tissue model. The ligand [^{11}C]-DASB (3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile) seems today to be the most widely used, because of its selectivity, reversibility, reliability and great specific binding [3]. A thorough review of different 5-HTT ligands has been given by Meyer [4]. A summary of the characteristics for [^{11}C]-MADAM and [^{11}C]-DASB is given in table 1.

Lu AA21004 (1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine) is a novel compound currently in phase III testing as an antidepressant [5]. In recombinant cell lines, the multimodal antidepressant Lu AA21004 is a 5-HT$_3$ and 5-HT$_7$ receptor antagonist, 5-HT$_{1A}$ receptor agonist, 5-HT$_{1B}$ receptor partial agonist and inhibitor of the 5-HTT. In non-clinical studies, Lu AA21004 increases levels of noradrenaline, dopamine, acetylcholine and histamine [6]. The affinities for the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_3$ and 5-HT$_7$ receptors and the 5-HTT are all considered to be of clinical relevance and involved in the mechanism of action at therapeutic doses. In contrast to selective serotonin reuptake inhibitors (SSRIs) and serotonin and noradrenalin reuptake inhibitors (SNRIs), which mainly have affinity for the 5-HTT [and noradrenalin transporter (NAT) in case of SNRIs], Lu AA21004 has a broad receptor-binding profile. During the clinical development of Lu AA21004, two PET studies have been performed using 5-HTT ligands (either [^{11}C]-MADAM or [^{11}C]-DASB) to quantify the 5-HTT occupancy in the brain of human subjects.

The purpose of this study was to describe the 5-HTT human brain occupancy after Lu AA21004 administration and its relation to the plasma concentrations of Lu AA21004.

Material and Methods

Study designs. Data originate from two different open-label studies, performed at different sites (necessary because of subject recruitment) and with access to different 5-HTT ligands. In study A, PET measurements were taken on day 1 (i.e. after a single dose) and on day 9 of multiple dosing in 11 young healthy Caucasian subjects using the [^{11}C]-MADAM ligand. In study B, PET measurements were taken after 13 days of multiple dosing in 35 young healthy Caucasian or Japanese subjects with the [^{11}C]-DASB ligand. Study A investigated the 5-HTT occupancy at three dose levels of Lu AA21004 (2.5, 10 and 60 mg/day), whereas the doses in study B were 2.5, 5 and 20 mg/day. Both studies were approved by local ethics committees and were conducted in accordance with Good Clinical Practice (GCP) and the Declaration of Helsinki.

Author for correspondence: Johan Areberg, H. Lundbeck A/S, Department of Clinical Pharmacology and Pharmacokinetics, Ottiliavej 9, DK-2500 Valby, Denmark (fax +4536438272, e-mail joar@lundbeck.com).

Table 1.
Characteristics of the PET ligands [^{11}C]-MADAM and [^{11}C]-DASB.

Parameter	[^{11}C]-MADAM	[^{11}C]-DASB
Specific-to-non-specific ratio	4.7 (raphe nuclei)[1]	2.04 ± 0.44 (midbrain)[2]
Plasma-free fraction	40%[1]	8.9 ± 1.6%[2]
Test–retest variability	0 ± 20% (raphe nuclei)[3]	1.7 ± 0.3% (raphe nuclei)[4]
SERT affinity, K_d (nM)	0.13 (monkey cortex)[5]	0.25 (monkey cortex)[5]

[1]Lundberg *et al.* [2]; [2]Frankle *et al.* [11]; [3]Lundberg *et al.* [12]; [4]Praschak-Rieder *et al.* [13]; [5]Elfving *et al.* [14].

Subjects

The data set consisted of 46 healthy male subjects (28 Caucasian and 18 Japanese) with a mean age of 28 years (range, 21–41). The mean weight and BMI were 73 kg (range, 57–95) and 23 kg/m^2 (range, 19–29), respectively. Subjects were enroled after they signed the informed consent form, and it was verified that subjects fulfilled the inclusion criteria and did not meet any exclusion criteria.

PET measurements. For each subject, two or three PET scans were performed: one at baseline (i.e. prior to Lu AA21004 dosing) and one or two during Lu AA21004 treatment. The PET ligands were administered as intravenous bolus injections prior to the start of the PET scan. The PET ligands for the scans during Lu AA21004 treatment were administered 7 hr after dosing of Lu AA21004 in both studies (at the approximate time for peak plasma concentration). Approximately 300 MBq (range, 153–320) of [^{11}C]-MADAM (study A) or approximately 370 MBq (range, 281–460) of [^{11}C]-DASB (study B) was administered intravenously to the subjects prior to each PET scan. The radioactivity in brain tissue was measured as a dynamic study over 90 min. with a Siemens ECAT Exact HR PET system (study A) or with a GE Discovery RX PETCT (GE Healthcare, Waukesha, WI, USA) system (study B), with the raphe nuclei as the region of interest (ROI). Both systems measured radioactivity in 47 brain sections with a centre-to-centre distance of 3.13 mm (study A) or 3.27 mm (study B). To determine the exact cerebral structures corresponding to the PET images, a standard 3D MRI scan was obtained in each subject immediately prior to the PET scans. Binding potential (BP) was derived from time–activity uptake curves (determined from PET using established methods) using the simplified reference tissue method, with the cerebellum as the reference region. Occupancy of the 5-HTT was calculated as

$$\text{Occupancy}(\%) = (\text{BP}_{\text{baseline}} - \text{BP}_{\text{treatment}})/\text{BP}_{\text{baseline}} * 100 \quad (1)$$

where $\text{BP}_{\text{baseline}}$ is the BP for the baseline scan and $\text{BP}_{\text{treatment}}$ is the BP after administrations of Lu AA21004.

Pharmacokinetic blood sampling. For study A, venous blood samples (2 mL) for the analysis of plasma content of Lu AA21004 were drawn on day 1 at 1, 2, 3, 4 and 6 hr post-dose; on days 2–8 at pre-dose; and on day 9 at 1, 2, 3, 4, 6, 7, 7.5, 8, 8.5, 9, 9.5, 10, 12, 14, 24, 36, 48, 72, 96 and 120 hr post-dose. For study B, samples were drawn on day 1 at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hr post-dose, on days 3, 5, 7, 9, 11 and 13 at pre-dose and on day 14 at 1, 2, 3, 4, 6, 7, 8, 10, 12, 24, 36, 48, 72, 96, 120, 144 and 168 hr post-dose. The plasma concentrations of Lu AA21004 were determined using an analytical method validated in accordance with FDA guidance [7]. Lu AA21004 was extracted from plasma followed by liquid chromatography and quantified by a mass spectrometry.

Data analysis. The relationship between the plasma concentration of Lu AA21004 at the start of the PET scan and the 5-HTT occupancy was investigated using non-linear regression analysis with an E_{max} model [8],

$$\text{Occupancy}(\%) = E_{max} * C_{PET}/(EC_{50} + C_{PET}) \quad (2)$$

where E_{max} is the maximal 5-HTT occupancy, C_{PET} the plasma concentration of Lu AA21004 at the start of the PET scan and EC_{50} the plasma concentration of Lu AA21004 giving rise to half E_{max} (potency). The analysis was performed using the software NONMEM® version 7 from ICON Development Solutions. The impact of the covariates PET ligand ([^{11}C]-MADAM or [^{11}C]-DASB) and race (Caucasian/Japanese) was tested by stepwise adding/deleting the covariates to the parameters E_{max} and EC_{50} in the model using a significance level of 0.01, which results in a decrease in the objective function value (equal to −2 * log likelihood) of 7.88. Descriptive statistics and plot drawings were performed using the software Tibco Spotfire S+, version 8.1 (TIBCO Software Inc, Palo Alto, California, US).

Results

The BP at baseline for [^{11}C]-MADAM had a mean (±S.D.) of 4.3 ± 1.7 and hence a coefficient of variation of 40%. For [^{11}C]-DASB, the BP at baseline had a mean (±S.D.) of 2.2 ± 0.39 and a coefficient of variation of 18%. The 5-HTT occupancies at different dosing regimens of Lu AA21004 administration (single or multiple doses) are shown in

Table 2.
5-HTT occupancy (mean ± S.D.) in the raphe nuclei for different doses of Lu AA21004, given as SD or MD.

Dose (mg)	Administration	Ligand	Number of subjects[1]	Occupancy (%)
2.5	SD	[^{11}C]-MADAM	4	27 ± 9.1
10	SD	[^{11}C]-MADAM	4	44 ± 7.5
60	SD	[^{11}C]-MADAM	3	70 ± 16
2.5	MD	[^{11}C]-MADAM,	4	35 ± 10
10	MD	[^{11}C]-MADAM	4	63 ± 23
60	MD	[^{11}C]-MADAM	3	93 ± 8.9
2.5	MD	[^{11}C]-DASB	12	49 ± 12
5	MD	[^{11}C]-DASB	11	51 ± 10
20	MD	[^{11}C]-DASB	12	90 ± 6.0

PET, positron emission tomography; SD, single dose; MD, multiple doses.
[1]Subjects given [^{11}C]-MADAM had PET measurements both single and multiple dosing, while subjects given [^{11}C]-DASB had PET measurements only after multiple dosing.

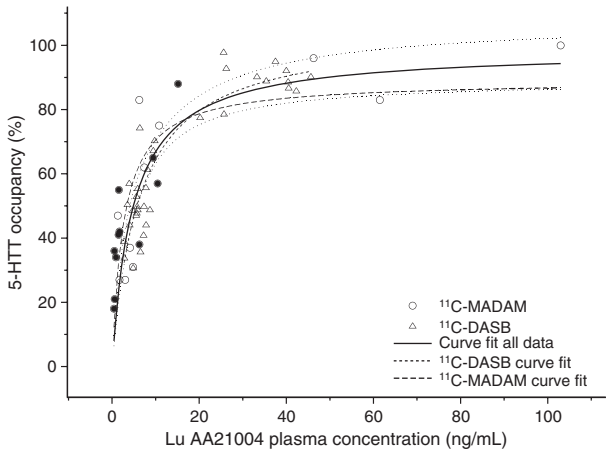

Fig. 1. The relationship between Lu AA21004 plasma concentration and 5-HTT occupancy. The plasma concentration of Lu AA21004 *versus* the observed (circles and triangles) and the estimated curve fits of the 5-HTT occupancy in the raphe nuclei region. The 95% confidence limits of the fitted curve are shown as dotted lines. Filled circles show single-dose data, while open circles and triangles show multiple-dose data.

table 2. The non-linear regression analysis relating plasma concentrations of Lu AA21004 and 5-HTT occupancy using the E_{max} model gave parameter values of 99% for E_{max} and 4.8 ng/mL for EC_{50}. The relative standard errors (standard error divided by mean value) were 3.8% and 17%, respectively. The nature of the PET ligand had no significant impact on E_{max} or EC_{50}, because the decrease in the objective function values was smaller than 7.88 for both ligands. This can also be observed in fig. 1, where the individual curve fits for two ligands are very close to each other. Likewise, race (Caucasian/Japanese) had no significant impact on E_{max} or EC_{50}. In fig. 1, the plasma concentrations of Lu AA21004 are plotted against the 5-HTT occupancy values.

Discussion

The mean BPs at baseline were 4.3 and 2.2 for [^{11}C]-MADAM and [^{11}C]-DASB, respectively. However, there were no significant differences in the Lu AA21004 plasma concentration-5-HTT occupancy relationship between two ligands. Neither E_{max} nor EC_{50} was influenced by which PET ligand had been used. This is consistent with a study by Elfving *et al.* [9], showing that the DASB and MADAM ligands had similar affinity for 5-HTT in rat and monkey brains. MADAM and DASB displayed significant specific binding to 5-HTT in monkey cerebellum, with receptor density (B_{max}) cortex/cerebellum ratios of 3 and 4 for MADAM and DASB, respectively. In rat brain, the B_{max} cortex/cerebellum ratios were 6 and 3 with MADAM and DASB, respectively [6].

It has been proposed that the occupancy of the 5-HTT should be around 80% for a conventional SSRI/SNRI to be therapeutically useful [4]. Lu AA21004 has been shown to be efficacious at doses of 5 and 10 mg/day in a clinical phase II proof-of-concept study in patients with major depressive disorder [10]. The plasma concentrations of Lu AA21004 at steady state for 5 mg correspond, using the relation between Lu AA21004 exposure and 5-HTT occupancy, to the 5-HTT occupancy of approximately 50%. This indicates that Lu AA21004 may be efficacious at lower 5-HTT occupancy than necessary with SSRI/SNRIs. The explanation for this may be Lu AA21004's multimodal neurotransmitter enhancer profile, where the affinity for the receptor subtypes 5-HT_{1A}, 5-HT_{1B}, 5-HT_3 and 5-HT_7 can contribute to the clinical effect.

In conclusion, we have shown that Lu AA21004 occupies the 5-HTT and that the relationship between plasma exposure of Lu AA21004 and 5-HTT occupancy can be reliably described by an E_{max} model.

Conflicts of interest

All authors are employed by H. Lundbeck A/S, the sponsor of two studies.

Disclosures

The authors have nothing to disclose.

References

1 Szabo Z, Kao PF, Scheffel U, Suehiro M, Mathews WB, Ravert HT *et al.* Positron emission tomography imaging of serotonin transporters in the human brain using [11C](+)McN5652. Synapse 1995;**20**:37–43.
2 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**:1505–15.
3 Meyer JH, Wilson AA, Ginovart N, Goulding V, Hussey D, Hood K *et al.* Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [11C]DASB PET imaging study. Am J Psychiatry 2001;**158**:1843–9.
4 Meyer JH. Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. Rev Psychiatr Neurosc 2007;**32**:86–102.
5 Bang-Andersen B, Ruhland T, Jørgensen M, Smith G, Frederiksen K, Jensen KG *et al.* Discovery of 1-[2-(2,4-dimethylphenylsulfanyl)phenyl]piperazine (Lu AA21004): a novel multimodal compound for the treatment of major depressive disorder. J Med Chem 2011;**54**:3206–21.
6 Moore NA, Bang-Andersen B, Brennum LT, Frederiksen K, Hogg S, Mørk A *et al.* Lu AA21004: a novel potential treatment for mood disorders. Eur Neuropsychopharmacol 2008;**18**(Suppl. 4):S321.
7 United States Food and Drug Administration (FDA). Guidance for industry. Bioanalytical method validation. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdfm. May 2001 (last accessed on October 2011).
8 Catafau AM, Perez V, Plaza P, Pascual J-C, Bullich S, Marina Suarez M *et al.* Serotonin transporter occupancy induced by paroxetine in patients with major depression disorder: a ^{123}I-ADAM SPECT study. Psychopharmacology 2006;**189**:145–53.
9 Elfving B, Madsen J, Knudsen G-M. Neuroimaging of the serotonin reuptake site requires high-affinity ligands. Synapse 2007;**61**:882–8.
10 Artigas F, Dragheim M, Loft H, Perez V, Alvarez E. A randomised, double-blind, placebo-controlled, active-referenced study of Lu AA21004 in patients with major depression. Eur Neuropsychopharmacol 2009;**19**(Suppl. 3):S426–7.

11 Frankle WG, Huang Y, Hwang DR, Talbot PS, Slifstein M, Van Heertum R *et al.* Comparative evaluation of serotonin transporter radioligands 11C-DASB and 11C-McN 5652 in healthy humans. J Nucl Med 2004;**45**:682–94.
12 Lundberg J, Halldin C, Farde L. Measurement of serotonin transporter binding with PET and [11C]MADAM: a test-retest reproducibility study. Synapse 2006;**60**:256–63.
13 Praschak-Rieder N, Wilson AA, Hussey D, Carella A, Wei C, Ginovart N *et al.* Effects of tryptophan depletion on the serotonin transporter in healthy humans. Biol Psychiatry 2005;**58**: 825–30.
14 Elfving B, Madsen J, Knudsen GM. Neuroimaging of the serotonin reuptake site requires high-affinity ligands. Synapse 2007; **61**:882–8.