REGULAR RESEARCH ARTICLE

Venlafaxine ER Blocks the Norepinephrine Transporter in the Brain of Patients with Major Depressive Disorder: a PET Study Using [$^{18}$F]FMenER-D$_2$

Ryosuke Arakawa, Per Stenkrona, Akihiro Takano, Jonas Svensson, Max Andersson, Sangram Nag, Yuko Asami, Yoko Hirano, Christer Halldin, Johan Lundberg

Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet, & Stockholm Health Care Services, Stockholm County Council, Stockholm, Sweden (Drs Arakawa, Stenkrona, Takano, Svensson, Andersson, Nag, Halldin, Lundberg); Central Nervous System, Medical Affairs, Pfizer Essential Health, Pfizer Japan Inc., Tokyo, Japan (Dr Asami and Ms Hirano).

Correspondence: Ryosuke Arakawa, MD, PhD, Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet, & Stockholm Health Care Services, Stockholm County Council, Stockholm, Sweden. Post address: Karolinska University Hospital Solna, R5:02, SE-17176 Stockholm, Sweden (ryosuke.arakawa@ki.se).

Abstract

Background: The in vivo binding of clinical dose of venlafaxine on norepinephrine transporter has been questioned because venlafaxine has higher in vitro affinity to serotonin transporter than that to norepinephrine transporter. Although serotonin transporter occupancy of clinically relevant doses of venlafaxine has been reported, there has been no report of norepinephrine transporter occupancy in the human brain.

Methods: This was an open-label, single center, exploratory positron emission tomography study. Twelve major depressive disorder patients who had responded to venlafaxine extended-release and 9 control subjects were recruited. Each subject participated in one positron emission tomography measurement with [$^{18}$F]FMenER-D$_2$. Binding potential in brain was quantified by the area under the curve ratio method with thalamus as target and white matter as reference regions. The difference of binding potential values between control and patient groups divided to 2 dose ranges were evaluated. Norepinephrine transporter occupancy (%) for all the major depressive disorder patients was calculated using mean binding potential of control subjects as baseline. The relationships between dose or plasma concentration of total active moiety and occupancies of norepinephrine transporter were also estimated.

Results: The binding potential of the patient group with 150 to 300 mg/d was significantly lower than that in the control subjects group ($P = .0004 < .05/2$). The norepinephrine transporter occupancy (8–61%) increased in a dose-dependent manner although a clear difference beyond 150 mg/d was not observed.

Conclusions: This study demonstrates that clinically relevant doses of venlafaxine extended-release block the norepinephrine transporter of the major depressive disorder patient's brain. The data support the notion that the antidepressant effect of venlafaxine involves a combination of serotonin transporter and norepinephrine transporter blockades.
Significance Statement

Based on in vitro data, venlafaxine is classified as a serotonin norepinephrine reuptake inhibitor. However, the in vivo inhibitory effect of clinical doses of venlafaxine on norepinephrine transporter (NET) has been questioned as the in vitro affinity for the serotonin transporter (5-HTT) is 2 orders of magnitude higher than for NET. Thus, we used positron emission tomography to investigate the NET occupancy of clinically relevant doses of venlafaxine extended-release (ER) in the living human brain of patients with major depressive disorder. This study demonstrates for the first time to our knowledge that clinically relevant doses of venlafaxine ER block the NET dose dependently in the human brain in vivo. The data support the notion that the antidepressant effect of venlafaxine involves blockade of both 5-HTT and NET.

Keywords: major depressive disorder, norepinephrine transporter, occupancy, positron emission tomography, venlafaxine ER

Introduction

Major depressive disorder (MDD) represents a major unmet medical need. For example, MDD was the number one cause of years lived with disability in 2015 in the world (World Health Organization, 2017). The development of better treatment options is one way to decrease its impact on global health. It has repeatedly been shown in the living human brain that subjects with MDD in many, but not all cases, show changes in expression levels of markers for the serotonin (5-HT) system, e.g., the serotonin transporter (5-HTT) (Savitz and Drevets, 2013; Gryglewski et al., 2014). In line with this observation, drugs that inhibit the reuptake of 5-HT such as selective serotonin reuptake inhibitors (SSRIs) have repeatedly proven efficacious for the treatment of MDD in many but not all subjects (Jakuowski et al., 2016; Locher et al., 2017; Cipriani et al., 2018). For example, in the STAR*D trial, the remission rate in 2876 MDD patients treated with SSRI was 30% (Trivedi et al., 2006). Another suggested antidepressant target is the norepinephrine transporter (NET). Postmortem data, and recently also in vivo data, suggest changes in NET densities in MDD subjects (Klimek et al., 1997; Moriguchi et al., 2017b). Indeed, it has been reported that dual action of a serotonergic and noradrenergic agent is associated with better clinical outcome in patients with MDD than single-action antidepressants (Nelson et al., 1991, 2004). Also, MDD patients who failed to respond to SSRIs have been shown to improve when switched to serotonin and norepinephrine reuptake inhibitors (SNRIs), which have dual-target sites including 5-HTT and NET (Papakostas et al., 2008; Perahia et al., 2008). On the other hand, a recent report shows no clear difference of the efficacy between SSRI and SNRI (Cipriani et al., 2018).

The 5-HTT occupancy has been highlighted as a measure of antidepressant treatment effect (Meyer et al., 2001, 2004; Suhara et al., 2003; Lundberg et al., 2012). But the fact that MDD biology commonly involves changes in NET densities, as well as the additive effect of NET blockade in antidepressant treatment, motivates further examination of NET occupancy in vivo in SNRI treatment. $^{[18F]}$FMeNER-D, is a positron emission tomography (PET) radioligand that binds reversibly and selectively to NET (Schou et al., 2004). A method for reliable quantification of $^{[18F]}$FMeNER-D binding to NET in human in vivo using PET has been described (Arakawa et al., 2008). The applicability of $^{[18F]}$FMeNER-D in clinical occupancy studies have been shown in several reports (Sekine et al., 2010; Nogami et al., 2013; Nyberg et al., 2013; Takano et al., 2014; Moriguchi et al., 2017a).

Venlafaxine, which was clinically introduced as the first SNRI for the treatment of MDD in 1993, has been widely and globally prescribed and its major prescribed formulation is the extended release (ER) (Thase et al., 2017). The in vitro affinity of venlafaxine has been reported to be higher for 5-HTT than for NET (Ki: 82 nM and 2480 nM, respectively) (Bymaster et al., 2001). Due to the large discrepancy of the affinity between NET and 5-HTT, the in vivo binding of clinical doses of venlafaxine on NET has been questioned, especially in the lower dose range (Koch et al., 2003). In a previous nonhuman primate (NHP) PET study, similar in vivo occupancy between 5-HTT and NET of venlafaxine has been demonstrated (Takano et al., 2013). While 5-HTT occupancy of clinically relevant doses of venlafaxine in the human brain has been reported previously (Meyer et al., 2004; Voineskos et al., 2007; Lundberg et al., 2012), there has been no report of in vivo NET occupancy of venlafaxine in the human brain.

Thus, the aims of this study were to verify that clinically relevant doses of venlafaxine ER occupy NET in the living human brain, and to identify the relationship between oral dose and plasma concentration of venlafaxine ER and NET occupancy in patients with MDD using PET and $^{[18F]}$FMeNER-D$_2$. Methods

Subjects

This was an open-label, single-center, exploratory PET study. Approval was obtained from the Regional Ethical Review Board in Stockholm, Sweden; the Radiation Safety Committee at the Karolinska University Hospital Solna in Stockholm, Sweden; and the Swedish Medical Product Agency (EudrACT 2016-004590-40). Oral and written informed consent was obtained from all participants after thorough oral and written information of the study, and before any study related activity took place.

Twelve MDD patients (age range, 22–65 years; mean ± SD, 37.4 ± 11.7; 6 males, 6 females) who had responded to venlafaxine ER (37.5–300 mg/d) treatment were recruited from Northern Stockholm Psychiatry, Stockholm Health Care Services. The response to the treatment was judged by clinical observations. Nine healthy volunteers (20–62 years; 39.9 ± 14.4 (P = .66 compared with patient group); 3 males, 6 females) were recruited as control subjects through advertisement in local newspapers and social media. Patients were diagnosed with MDD based on the Diagnostic and Statistical manual of Mental Disorders, 4th edition (DSM-IV-TR). The dose of venlafaxine ER had been fixed for more than 2 weeks before PET measurement. The MDD patients did not take any other antidepressants or psychotropic agents or any other medication that might influence 5-HT and NE transmission for at least 4 weeks before PET measurement.
In addition, the MDD patients did not take structured psychotherapy or behavioral therapy for at least 3 months before the PET measurement. Exclusion criteria for patients and controls included past psychiatric (with the exception of MDD in the patients), neurological, or somatic disorders, or alcohol- or drug-related problems. Habitual nicotine use within 3 months prior to the PET examination was also an exclusion criterion. All subjects were healthy according to somatic and psychiatric interview (apart from MDD in the patient group), somatic examination, 12-lead electrocardiography, and blood and urine tests. A pregnancy test was done for female subjects using a urine pregnancy strip test.

PET Procedures

Each subject participated in one PET measurement with [18F] FMeNER-D2. For MDD patients, the last administration of venlafaxine ER was approximately 5 hours before radioligand injection. A plastic helmet was used during the PET measurement to minimize head movement. [18F]FMeNER-D2 was prepared as previously reported (Schou et al., 2004). After a bolus injection (<10 seconds) of the radioligand, the emission data were collected from 120 to 180 minutes using a HRRT system (Siemens Molecular Imaging). The injected radioactivity was adjusted by body weight. For an attenuation correction, a 6-minute transmission using a single 137Cs source was also performed. The data were reconstructed using the ordinary Poisson-3D-ordered subset expectation maximization (OP-3D-OSEM) algorithm with 10 iterations and 16 subsets including modeling of the point spread function (Varrone et al., 2009). T1-weighted magnetic resonance imaging (MRI) was also performed for the anatomical reference (Arakawa et al., 2008; Owens et al., 2008; Takano et al., 2008; Moriguchi et al., 2017a). The mean BPND of control subjects was used as the BPND of MDD patients, which allows the comparison of the PET procedures of the control and patient groups (Arakawa et al., 2008; Takano et al., 2008; Moriguchi et al., 2017a). To select the reference region, the difference of standardized uptake value (SUV) of each patient relative to averaged SUV of control subjects was calculated for the 2 previously evaluated reference regions: caudate (Arakawa et al., 2008; Takano et al., 2008) and white matter (Takano et al., 2008; Moriguchi et al., 2017a). Then the SUV difference for all patients was compared between caudate and white matter using a paired t test. The reference showing smaller difference was chosen. The difference of BPND values between control subjects (n = 9) and patients (n = 7) was calculated as (1) Omax was also estimated as well as Kd.

Plasma Concentration of Venlafaxine and O-Desmethylvenlafaxine

For all MDD patients, 3 venous blood samplings were performed before and 120 and 180 minutes after the radioligand injection to measure the plasma concentration of venlafaxine and its main active metabolite, O-desmethylvenlafaxine. Samples were analyzed using a validated high performance liquid chromatography-tandem mass-spectrometry method at WuXi AppTec Co. Ltd. (Shanghai, China). In this study, total active moieties as sum of venlafaxine and O-desmethylvenlafaxine was chosen as the parameter of interest as reported elsewhere (Hynninen et al., 2008; Owens et al., 2008; Takano et al., 2013). Averaged plasma concentration using a trapezoidal method during 180 minutes, from PET radioligand injection to end of PET measurement, was used for further analysis.

Data Analysis

Anatomical regions of interest (ROIs) were delineated on the reoriented MRI image using the Automated Anatomical Labelling template using Matlab7.5 toolbox (MathWorks Inc.) and SPM5 (Wellcome Trust Centre for Neuroimaging). White matter was defined using SPM5 segmentation. MRI and ROIs were co-registered to summed PET image with the mutual information algorithm using SPM5. Time activity curves were obtained from co-registered ROIs on dynamic PET images. [18F]FMeNER-D2 binding potential (BPND) in brain was quantified using a trapezoidal method during 180 minutes, which was concentration using a trapezoidal method during 180 minutes, the emission data were collected from 120 to 180 minutes using a HRRT system (Siemens Molecular Imaging). The injected radioactivity was adjusted by body weight. For an attenuation correction, a 6-minute transmission using a single 137Cs source was also performed. The data were reconstructed using the ordinary Poisson-3D-ordered subset expectation maximization (OP-3D-OSEM) algorithm with 10 iterations and 16 subsets including modeling of the point spread function (Varrone et al., 2009). T1-weighted magnetic resonance imaging (MRI) was also performed for the anatomical reference (Arakawa et al., 2008; Owens et al., 2008; Takano et al., 2008; Moriguchi et al., 2017a). The mean BPND of control subjects was used as the BPND of MDD patients, which allows the comparison of the PET procedures of the control and patient groups (Arakawa et al., 2008; Takano et al., 2008; Moriguchi et al., 2017a). The mean BPND of control patients was used as the BPND of MDD patients, which allows the comparison of the PET procedures of the control and patient groups (Arakawa et al., 2008; Takano et al., 2008; Moriguchi et al., 2017a). The mean BPND of control subjects was used as the BPND of MDD patients, which allows the comparison of the PET procedures of the control and patient groups (Arakawa et al., 2008; Takano et al., 2008; Moriguchi et al., 2017a). To select the reference region, the difference of standardized uptake value (SUV) of each patient relative to averaged SUV of control subjects was calculated for the 2 previously evaluated reference regions: caudate (Arakawa et al., 2008; Takano et al., 2008) and white matter (Takano et al., 2008; Moriguchi et al., 2017a). Then the SUV difference for all patients was compared between caudate and white matter using a paired t test. The reference showing smaller difference was chosen. The difference of BPND values between control subjects (n = 9) and patients group divided into 2 dose ranges (low dose: 37.5–75 mg/d and high dose: 150–300 mg/d; each n = 6) was evaluated using Wilcoxon rank sum test. Statistical significance was set as P < .025 (= .05/2) using Bonferroni correction. To create averaged BPND images of control and patient groups, spatial normalization by SPM5 was applied to each subject's BPND image.

NET Occupancy

NET occupancy (%) was calculated as BPND (baseline − BPND (maximum)) / BPND (baseline) × 100 (Nogami et al., 2013; Takano et al., 2014; Moriguchi et al., 2017a). The mean BPND of control subjects was used as the BPND of MDD patients, which allows the comparison of the PET procedures of the control and patient groups (Arakawa et al., 2008; Takano et al., 2008; Moriguchi et al., 2017a). The mean BPND of control subjects was used as the BPND of MDD patients, which allows the comparison of the PET procedures of the control and patient groups (Arakawa et al., 2008; Takano et al., 2008; Moriguchi et al., 2017a). To select the reference region, the difference of SUV of each patient relative to averaged SUV of control subjects was calculated for the 2 previously evaluated reference regions: caudate (Arakawa et al., 2008; Takano et al., 2008) and white matter (Takano et al., 2008; Moriguchi et al., 2017a). Then the SUV difference for all patients was compared between caudate and white matter using a paired t test. The reference showing smaller difference was chosen. The difference of BPND values between control subjects (n = 9) and patients group divided into 2 dose ranges (low dose: 37.5–75 mg/d and high dose: 150–300 mg/d; each n = 6) was evaluated using Wilcoxon rank sum test. Statistical significance was set as P < .025 (= .05/2) using Bonferroni correction. To create averaged BPND images of control and patient groups, spatial normalization by SPM5 was applied to each subject's BPND image.

RESULTS

Injected radioactivity was 285 ± 52 (mean ± SD) MBq and 268 ± 53 MBq for MDD patients and control subjects, respectively. Molar radioactivity at the time of injection was 64 ± 27 GBq/µmol and 68 ± 32 GBq/µmol, and injected mass was 1.7 ± 0.7 µg and 1.5 ± 0.6 µg, respectively. There was no statistical difference between MDD patients and control subjects for any of the 3 parameters (Student’s t test: P = .47–.76).

White matter showed a smaller difference than caudate (0.12 vs 0.15; P = .07) in SUV of the patient relative to control group. Therefore, white matter was used as reference region in the further analysis. Daily doses of venlafaxine ER varied between 37.5 and 300 mg/d, plasma concentrations of total active moieties between 108 and 947 ng/mL, and NET occupancy in thalamus between 8% and 61% for all MDD patients (Table 1). Averaged BPND images of controls and patients for 2 dose ranges are shown in Figure 1. BPND in the thalamus showed an inverse relation to the dose of venlafaxine ER. The BPND of the 150- to 300-mg/d patient group was significantly lower than that of the control subjects group (P = .0004), whereas it was not significant in the patient group with 37.5 to 75 mg/d (P = .07) (Figure 2).
The relationship between dose of venlafaxine ER and NET occupancy is shown in Figure 3. The relationship between plasma concentration of total active moiety and NET occupancy is shown in Figure 4. The NET occupancy increased in a dose- and plasma concentration-dependent manner, although no obvious escalation was observed in higher range. $K_d$ of dose ($K_d$:dose) and plasma concentration ($K_d$:conc) was 248 mg/d and 671 ng/mL, respectively, with fixed 100% $O_{max}$. When fitting $O_{max}$, $K_d$:dose and $K_d$:conc were 130 mg/d and 246 ng/mL, respectively, and $O_{max}$ of dose ($O_{max}$:dose) and plasma concentration ($O_{max}$:conc) were 71% and 62%, respectively.

### Discussion

In this study, we demonstrated that the NET occupancy in MDD patients who had responded to clinically relevant doses (37.5–300 mg/d) of venlafaxine ER was 8% to 61%, increasing in a dose- and plasma concentration-dependent manner. The NET $BP_{ND}$ in patients with MDD compared with controls decreased significantly with higher doses (150 mg/d or more) of venlafaxine ER, whereas no significant differences were detected at lower doses (75 mg/d or less). Several PET studies have demonstrated that 5-HTT occupancy of patients with MDD who were treated with antidepressants was 65% to 80% (Meyer et al., 2001, 2004; Suhara et al., 2003; Lundberg et al., 2012). It has been reported that 5-HTT occupancy of venlafaxine at 75 mg/d already reaches 80% with a plateau for higher doses (Meyer et al., 2004). Therefore, the NET occupancy that we report here at doses 150 mg/d or higher in combination with the 5-HTT occupancy previously shown is one of possible explanations for the clinical efficacy of venlafaxine in the treatment of MDD (Rudolph et al., 1998; Charlier et al., 2002; Linden et al., 2003; Thase et al., 2006). However, there was no clear difference in NET occupancy between the 150 mg/d (n = 2) and higher (187.5–300 mg/d; n = 4) doses in the present study. The question whether the reported higher response rate in doses of 225 or 375 mg/d (Rudolph et al., 1998; Thase et al., 2006) may be explained by even higher NET occupancy than at 150 mg/d should be addressed in a PET study designed and powered for this specific purpose.

Meyer et al. have reported the oral dose associated with 50% occupancy (the apparent dose-related affinity, $K_{d}$:dose) of venlafaxine ER in human in vivo for 5-HTT to be 5.8 mg/d (Meyer et al., 2004). $K_{d}$:dose of venlafaxine ER for NET in the present study was

---

**Table 1. Age, gender, dose of venlafaxine ER, plasma concentration, and NET occupancy for MDD patients**

| Subject | Age (years) | Gender | Dose (mg/d) | Plasma concentration (ng/mL) | NET occupancy (%) |
|---------|-------------|--------|-------------|-------------------------------|------------------|
| 1       | 37          | F      | 37.5        | 18                            | 117              | 10               |
| 2       | 65          | F      | 37.5        | 89                            | 42               | 18               |
| 3       | 22          | M      | 37.5        | 21                            | 91               | 23               |
| 4       | 41          | M      | 75          | 38                            | 117              | 8                |
| 5       | 28          | M      | 75          | 28                            | 94               | 8                |
| 6       | 34          | M      | 75          | 25                            | 83               | 36               |
| 7       | 47          | F      | 150         | 163                           | 267              | 54               |
| 8       | 44          | F      | 150         | 66                            | 268              | 334              |
| 9       | 37          | M      | 187.5       | 72                            | 272              | 344              |
| 10      | 32          | F      | 225         | 794                           | 153              | 947              |
| 11      | 23          | F      | 225         | 231                           | 649              | 880              |
| 12      | 33          | M      | 300         | 744                           | 72               | 816              |

ER, extended release; MDD, major depressive disorder; NET, norepinephrine transporter; ODV, O-desmethylvenlafaxine; VEN, venlafaxine.

---

**Figure 1.** Averaged binding potential ($BP_{ND}$) images of control subjects and major depressive disorder (MDD) patients for 2 dose ranges.
20 to 40 times higher (130 or 248 mg/d with or without fitting Omax). This ratio is in the same order of magnitude as the ratio of in vitro affinity for 5-HTT (82 nM) and NET (2480 nM) (Bymaster et al., 2001). The plasma concentration of venlafaxine associated with 50% occupancy (the apparent plasma concentration, Kd:conc) in human in vivo for 5-HTT has been reported to be 3.4 ng/mL (parent venlafaxine only) (Meyer et al., 2004). In the present study, Kd:conc for NET was 246 and 671 ng/mL (total active moiety as sum of venlafaxine and O-desmethylvenlafaxine), and 34 or 245 ng/mL (parent venlafaxine only; data not shown). In a previous PET study in NHP, it was demonstrated that the ratio of Kd:conc for 5-HTT (14.5 ng/mL) and NET (26.1 ng/mL) was 1.8 (Takano et al., 2013). Another study using a human ex vivo serum assay reported the ratio of Kd for 5-HTT (85 ng/mL) and NET (325 ng/mL) was 3.8 (Owens et al., 2008). Although there are numerical differences between methods, it has consistently been shown that venlafaxine has higher affinity for 5-HTT than NET.

So far, 5-HTT and NET occupancy in the human brain has been reported for 2 other SNRIs: milnacipran and duloxetine. Nogami et al. demonstrated 33% to 62% 5-HTT occupancy and 25% to 50% NET occupancy in MDD patients after administration of 25 to 200 mg/d milnacipran (Nogami et al., 2013). The Kd:dose was estimated to 122.5 mg for 5-HTT and 149.9 mg for NET. This relatively equivalent in vivo occupancy of 5-HTT and NET is in line with the relation of the in vitro affinity of milnacipran (Ki = 8.4/22 nM for 5-HTT/NET) (Vaishnavi et al., 2004). In addition, both 5-HTT and NET occupancy have been examined in control subjects after single oral doses of duloxetine. The 5-HTT occupancy after 5 to 60 mg of duloxetine was 44% to 82% and Kd:dose was estimated to 7.9 mg (Takano et al., 2006). The NET occupancy after 20 to 60 mg of duloxetine was 30% to 40% and Kd:dose was 76.8 mg (Moriguchi et al., 2017a). The difference between Kd:dose values of duloxetine for 5-HTT and NET corresponds to the difference in the reported in vitro affinity (Ki = 0.8/7.5 nM for 5-HTT/NET) (Bymaster et al., 2001). Our data suggest that NET occupancy from clinical doses of venlafaxine may be higher than those from milnacipran and duloxetine in human brain, although a direct comparison is difficult because of the differences in study design and target subjects.

Previous PET studies using reference tissue models to quantify[^1]FMeNER-D2 binding have applied either caudate (Arakawa et al., 2008; Takano et al., 2008) or white matter (Takano et al., 2008; Moriguchi et al., 2017a) as reference region. In the present analysis, we used white matter as reference region. Theoretically, the caudate is suitable reference for quantification of[^1]FMeNER-D2 because of its negligible density of NET. In the present analysis, we used white matter as reference region. Theoretically, the caudate is suitable reference for quantification of[^1]FMeNER-D2 because of its negligible density of NET.
ventricle, resulting in an unstable estimation of NET binding in target regions. The smaller difference in SUV between groups for white matter compared with caudate indicates that white matter reference should produce more valid occupancy estimates in this sample.

In this study, \(O_{\text{max}}\) was set to 100% as in previously published reports of NET occupancy in human brain (Nogami et al., 2013; Takano et al., 2014; Moriguchi et al., 2017b), and fitted as well as \(K_{\text{d}}\). When fitting \(O_{\text{max}}\), it was estimated <100% (71% and 62% for dose and plasma concentration, respectively). One possible reason is that relatively low occupancy (up to 60%) was obtained in this study. However, almost fully blocked NET was observed in previous NHP PET studies (Takano et al., 2009, 2013; Gallezot et al., 2011). We reported both results because there might not be strict reason to exclude any of the methods.

There are several limitations in this study. First, we used the average BP_{ND} of control subjects as an estimation of baseline BP_{ND} for all MDD patients. Recently, around 30% higher NET availabilities in unmedicated patients with MDD compared with control subjects were reported using \[^{[18]}\text{F}\]FMENER-D2 (19 patients vs 19 controls, \(P = .007\)) (Moriguchi et al., 2017b). Our patients were responders to the antidepressant treatment, and it is not clear whether an increased NET binding in MDD is a state or trait phenomenon. However, if the baseline NET binding is underestimated due to the study design, we may have underestimated the NET occupancy systematically in all the examined patients, so that in the present data set, where occupancy was calculated to between 8% and 61%, the true occupancy may have been between 29% and 70%.

Second, up- or downregulation of NET proteins by chronic administration of antidepressants has been reported in rodent brain using the autoradiography method (Bauer and Tejani-Butt, 1992; Hébert et al., 2001; Weinschenker et al., 2002; Benmansour et al., 2004). Although it is unclear if this phenomenon exists in MDD patients exposed to clinical doses of venlafaxine ER, we cannot rule out that this may have affected our estimation of NET occupancy.

Third, the relationship between the clinical efficacy and NET occupancy by venlafaxine ER could not be investigated with the present protocol, as this included one PET examination in MDD patients who had responded to venlafaxine treatment. To determine the lower limit of NET occupancy needed for clinical effect, a prospective study in unmedicated MDD patients would be needed.

Conclusions

This study demonstrates for the first time to our knowledge that clinically relevant doses of venlafaxine ER block the NET in the living human brain of MDD patients. Additionally, it is shown that the NET occupancy of venlafaxine varies in a dose- and plasma concentration-dependent manner. The present results are in line with the notion that venlafaxine ER exerts its clinical effect via blockade of both 5-HTT and NET.

Acknowledgments

We thank all members of the Karolinska Insitutet PET Centre for assistance with the PET experiments, and Mattias Agestam and Sead Omerov of Stockholm Health Care Services for help with patient recruitment. JS, MA, and JL were funded by grant 2013-09304 from the Swedish Research Council. This work was sponsored by Pfizer Japan Inc.

Statement of Interest

YA and YH are the employees of Pfizer Japan Inc. Other authors declare that they have no conflict of interest.

References

Arakawa R, Okumura M, Ito H, Seki C, Takahashi H, Takano H, Nakao R, Suzuki K, Okubo Y, Haldin C, Suhara T (2008) Quantitative analysis of norepinephrine transporter in the human brain using PET with \((S,S)-18F\text{-FMENER-D2}\). J Nucl Med 49:1270–1276.

Bauer ME, Tejani-Butt SM (1992) Effects of repeated administration of desipramine or electroconvulsive shock on norepinephrine uptake sites measured by \[^{[3]}\text{H}\]nisoxetine autoradiography. Brain Res 582:208–214.

Benmansour S, Altamirano AV, Jones DJ, Sanchez TA, Gould GG, Pardon MC, Morilak DA, Frazer A (2004) Regulation of the norepinephrine transporter by chronic administration of antidepressants. Biol Psychiatry 55:313–316.

Bymaster FP, Dreshfield-Ahmad LJ, Threlkeld PG, Shaw JL, Thompson L, Nelson DL, Hemrick-Luecke SK, Wong DT (2001) Comparative affinity of duloxetine and venlafaxine for serotonin and norepinephrine transporters in vitro and in vivo, human serotonin receptor subtypes, and other neuronal receptors. Neuropsychopharmacology 25:871–880.

Charlier C, Pinto E, Ansseau M, Plomteux G (2002) Venlafaxine: the relationship between dose, plasma concentration and clinical response in depressive patients. J Psychopharmacol 16:369–372.

Cipriani A, Furukawa TA, Salanti G, Chaimani A, Atkinson LZ, Ogawa Y, Leucht S, Ruhe HG, Turner EH, Higgins JPT, Egger M, Takeshima N, Hayasaka Y, Imai H, Shinohara K, Tajika A, Ioannidis JPA, Geddes JR (2018) Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. Lancet 391:1357–1366.

Gallezot JD, Weinzimmer D, Nabulsi N, Lin SF, Fowles K, Sandiego C, McCarthy TJ, Maguire RP, Carson RE, Ding YS (2011) Evaluation of \((11)\text{C}\)MRB for assessment of occupancy of norepinephrine transporters: studies with atomoxetine in non-human primates. Neuroimage 56:268–279.

Gyglewski G, Lanzenberger R, Kranz GS, Cumming P (2014) Meta-analysis of molecular imaging of serotonin transporters in major depression. J Cereb Blood Flow Metab 34:1096–1103.

Hébert C, Habimana A, Elie R, Reader TA (2001) Effects of chronic antidepressant treatments on 5-HT and NA transporters in rat brain: an autoradiographic study. Neurochem Int 38:63–74.

Hynninen VV, Olkkola KT, Bertilsson L, Kurkinen K, Neuvonen PJ, Laine K (2008) Effect of terbinafine and voriconazole on the pharmacokinetics of the antidepressant venlafaxine. Clin Pharmacol Ther 83:342–348.

Jakubowski E, Varigonda AL, Freemantle N, Taylor MJ, Bloch MH (2016) Systematic review and meta-analysis: dose-response relationship of selective serotonin reuptake inhibitors in major depressive disorder. Am J Psychiatry 173:174–183.

Klimek V, Stockmeier C, Overholser J, Meltzer HY, Kalka S, Dilley G, Ordway GA (1997) Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. J Neurosci 17:8451–8458.

Koch S, Hemrick-Luecke SK, Thompson LK, Evans DC, Threlkeld PG, Nelson DL, Perry KW, Bymaster FP (2003) Comparison of effects of dual transporter inhibitors on monoamine trans-
Porter's and extracellular levels in rats. Neuropharmacology 45:935–944.

Linden M, Ludewig K, Munz T, Dierkes W (2003) Dosage finding and outcome of venlafaxine treatment in psychiatric outpatients and inpatients: results of a drug utilization observation study. Pharmacopsychiatry 36:197–205.

Locher C, Koechlin H, Zion SR, Werner C, Fine DS, Kirsch I, Kessler RC, Kossowsky J (2017) Efficacy and safety of selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and placebo for common psychiatric disorders among children and adolescents: a systematic review and meta-analysis. JAMA Psychiatry 74:1011–1020.

Lundberg J, Tiger M, Landén M, Hallidin C, Farde L (2012) Serotonin transporter occupancy with TCAs and SSRIs: a PET study in patients with major depressive disorder. Int J Neuropsychopharmacol 15:1167–1173.

Meyer JH, Wilson AA, Ginovart N, Goulding V, Hussey D, Hood K, Houle S (2001) Occurrence of serotonin transporters by paroxetine and citalopram during treatment of depression: a [(11)C]DASB PET imaging study. Am J Psychiatry 158:1843–1849.

Meyer JH, Wilson AA, Sagrati S, Hussey D, Carella A, Potter WZ, Ginovart N, Spencer EP, Cheok A, Houle S (2004) Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [(11)C]DASB positron emission tomography study. Am J Psychiatry 161:826–835.

Moriguchi S, Takano H, Kimura Y, Nagashima T, Takahata K, Kubota M, Kitamura S, Ishii T, Ichiue M, Zhang MR, Shimada H, Mimura M, Meyer JH, Higuchi M, Suhara T (2017a) Occupancy of norepinephrine transporter by duloxetine in human brains measured by positron emission tomography with (S,S)-(18)F-FMeNER-D2. Int J Neuropsychopharmacol 20:957–962.

Moriguchi S, Yamada M, Takano H, Nagashima T, Takahata K, Yokokawa K, Ito T, Ishii T, Kimura Y, Zhang MR, Mimura M, Suhara T (2017b) Norepinephrine transporter in major depressive disorder: a PET study. Am J Psychiatry 174:36–41.

Nelson JC, Mazure CM, Bowers MB Jr, Jatlow PJ (1991) A preliminary, open study of the combination of fluoxetine and desipramine for rapid treatment of major depression. Arch Gen Psychiatry 48:303–307.

Nelson JC, Mazure CM, Jatlow PJ, Bowers MB Jr, Price LH (2004) Combining norepinephrine and serotonin reuptake inhibition mechanisms for treatment of depression: a double-blind, randomized study. Biol Psychiatry 55:296–300.

Nogami T, Takano H, Arakawa R, Ichimiya T, Inoue M, Yasuno F, Ikoma Y, Okubo Y (2003) High levels of serotonin transporter occupancy with low-dose clomipramine in comparative occupancy study with fluvoxamine using positron emission tomography. Arch Gen Psychiatry 60:386–391.

Takano A, Suzuki K, Kosaka J, Ota M, Nosaki I, Ikoma Y, Tanada S, Suhara T (2006) A dose-finding study of duloxetine based on serotonin transporter occupancy. Psychopharmacology (Berl) 185:395–399.

Takano A, Varrone A, Gulyás B, Karlsson P, Tauscher J, Hallidin C (2008) Mapping of the norepinephrine transporter in the human brain using PET with (S,S)-(18)F-FMeNER-D2. Neuroimage 42:474–482.

Takano A, Gulyás B, Varrone A, Maguire RP, Hallidin C (2009) Saturated norepinephrine transporter occupancy by atomoxetine relevant to clinical doses: a rhesus monkey study with (S,S)-(18)F-FMeNER-D2. Eur J Nucl Med Mol Imaging 36:1308–1314.

Takano A, Hallidin C, Farde L (2013) SERT and NET occupancy by venlafaxine and milnacipran in nonhuman primates: a PET study. Psychopharmacology (Berl) 226:147–153.

Takano H, Arakawa R, Nogami T, Suzuki M, Nagashima T, Fujiwara H, Kimura Y, Kodaka F, Sato T, Takahata K, Shimada H, Murakami Y, Tateno A, Yamada M, Ito H, Kawamura K, Zhang MR, Takahashi H, Kato M, Okubo Y, Suhara T (2014) Norepinephrine transporter occupancy by nortriptyline in patients with depression: a positron emission tomography study with (S,S)-(18)F-FMeNER-D3. Int J Neuropsychopharmacol 17:553–560.

Thase M, Asami Y, Wajsbrodt D, Dorries K, Boucher M, Pappadopoulos E (2017) A meta-analysis of the efficacy of venlafaxine extended release 75–225 mg/day for the treatment of major depressive disorder. Curr Med Res Opin 33:317–326.

Thase ME, Shelton RC, Khan A (2006) Treatment with venlafaxine extended release after SSRI nonresponse or intolerance: a randomized comparison of standard- and higher-dosing strategies. J Clin Psychopharmacol 26:250–258.

Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ, Shores-Wilson K, Biggs MM, Balasubramani GK, Fava M; STAR*D Study Team (2006) Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. Am J Psychiatry 163:28–40.
Vaishnavi SN, Nemeroff CB, Plott SJ, Rao SG, Kranzler J, Owens MJ (2004) Milnacipran: a comparative analysis of human monoamine uptake and transporter binding affinity. Biol Psychiatry 55:320–322.

Varrone A, Sjöholm N, Eriksson L, Gulyás B, Halldin C, Farde L (2009) Advancement in PET quantification using 3D-OP-OSEM point spread function reconstruction with the HRRT. Eur J Nucl Med Mol Imaging 36:1639–1650.

Voineskos AN, Wilson AA, Bouvissara A, Sagrati S, Houle S, Rusjan P, Sokolov S, Spencer EP, Ginovart N, Meyer JH (2007) Serotonin transporter occupancy of high-dose selective serotonin reuptake inhibitors during major depressive disorder measured with [11C]DASB positron emission tomography. Psychopharmacology (Berl) 193:539–545.

Weinshenker D, White SS, Javors MA, Palmiter RD, Szot P (2002) Regulation of norepinephrine transporter abundance by catecholamines and desipramine in vivo. Brain Res 946:239–246.

World Health Organization (2017) Depression and other common mental disorders: global health estimates. WHO/MSD/MER/20172. Available at: https://www.who.int/mental_health/prevalence_global_health_estimates/en/.