The role of dopamine and serotonin in cervical dystonia

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THE EFFECT OF ESCITALOPRAM AND PLACEBO ON CENTRAL SEROTONERGIC AND DOPAMINERGIC SYSTEMS IN PATIENTS WITH CERVICAL DYSTONIA, AND ITS RELATIONSHIP WITH CLINICAL TREATMENT EFFECTS

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In preparation
Chapter 6

ABSTRACT

Purpose: The pathophysiology of cervical dystonia (CD) is thought to be related to changes in dopamine and serotonin levels in the brain. We recently performed an escitalopram (SSRI) trial, with the aim to restore the imbalance in these neurotransmitters. Here, we report on changes in dopamine D_{2/3} receptor (D2/3R), dopamine transporter (DAT) and serotonin transporter (SERT) binding potential (BP_{ND}) after a 6 week treatment course with escitalopram or placebo.

Methods: Patients with CD had [^{123}I]FP-CIT SPECT scans, to quantify extrastriatal SERT and striatal DAT, and [^{123}I]IBZM SPECT scans to quantify striatal D2/3R BP_{ND} before and after 6 weeks of treatment with either escitalopram or placebo. Treatment effect was evaluated with the Clinical Global Impression scale for dystonia, jerks and psychiatric symptoms. Participating physicians rated video recordings and interviews, and patients scored their symptoms subjectively.

Results: Patients treated with escitalopram who reported a positive effect on dystonia or psychiatric symptoms had significantly higher SERT occupancy compared to patients who did not experience an effect. Comparing scans after treatment with escitalopram (n=8) to placebo (n=8) showed a trend (p=0.13) towards lower extrastriatal SERT BP_{ND} in the SSRI group (median SERT occupancy of 64.6%). No significant differences were detected after treatment in striatal D2/3R and DAT BP_{ND}. In patients treated with placebo there were also no significant differences in SERT, DAT or D2/3R BP_{ND}.

Conclusion: Treatment with escitalopram decreases SERT BP_{ND} in patients with CD. Higher extrastriatal SERT occupancy after treatment with escitalopram is associated with a positive subjective effect on dystonia and psychiatric symptoms in CD patients.
INTRODUCTION

Cervical dystonia (CD) is the most common form of focal dystonia. Besides dystonia of the neck muscles, patients often suffer from a variety of other motor symptoms, such as jerks or head tremor (around 50% of patients) and psychiatric symptoms, mainly depression and anxiety (lifetime prevalence between 40-90%).

The pathophysiology of CD and other forms of dystonia is not fully understood, but alterations in neurotransmitters such as dopamine and serotonin are hypothesized to play an important role. In line with this hypothesis, in two recent single-photon emission computed tomography (SPECT) studies in CD we have shown that abnormalities in central dopaminergic and serotonergic systems are mainly related to depressive symptoms. Furthermore, we found a positive correlation between striatal dopamine transporter (DAT) binding potential (BP_{ND}) and severity of myoclonus in CD measured with the Unified Myoclonus Rating Scale (UMRS).

Treatment with selective serotonin receptor inhibitors (SSRIs) is an effective pharmacological intervention for major depression. After short-term treatment with SSRIs (4-6 weeks), SERT binding in midbrain, thalamus and striatum is significantly reduced (40-70%) in depressed patients as well as healthy controls, representing occupancy of the SERT by the SSRI. Treatment of major depression with a therapeutic dose of different SSRIs led to midbrain SERT occupancy of around 80%, if measured shortly after ingestion of the last dosage. Increasing the SSRI dose leads to higher plasma levels but SERT occupancy does not further increase (ceiling effect). This is in line with the fact that increasing the dose of an SSRI does not increase treatment efficacy, but may lead to more side effects, likely (partly) caused by an effect of SSRIs on other neurotransmitter systems, such as the dopamine system. It was found that the striatal DAT binding is significantly increased by approximately 20% during a successful 6 week treatment course with an SSRI, although this effect is smaller in older patients. Altogether, there are strong indications that the therapeutic effect of SSRIs, at least in major depression, is related to a SERT occupancy of 80%.

In dystonia, the role of the serotonin system is far less clear than in major depression. However serotonergic abnormalities are likely to play a role in the pathophysiology of CD (for a recent review see Smit). Recently, we conducted a crossover trial with the SSRI escitalopram, to evaluate the efficacy in CD with jerks/tremor. In this particular study, we did not find an add-on SSRI treatment effect to botulinum neurotoxin (BoNT) comparing a 6 week treatment course with escitalopram or placebo. To examine SERT occupancy and investigate the relationship between SERT occupancy and clinical effect,
as well as to evaluate the effect of SSRI treatment in CD on the dopaminergic system, we studied the effect on both the dopamine and serotonin system in each subject, before and after treatment with serial SPECT scans. In the current article, we report the effects on DAT, D2/3 receptor (D2/3R) and SERT BPND as measured with SPECT, in relation to the clinical effect. We hypothesized that in CD patients treated with placebo tracer binding to DAT, D2/3R and SERT would remain unchanged. In CD patients treated with escitalopram we hypothesized that SERT binding would be significantly reduced (representing occupancy, expected around 80%) and D2/3R and DAT binding would be increased. Furthermore, we investigated whether SERT occupancy and change in D2/3R and DAT binding were related to clinical outcomes.

MATERIAL AND METHODS

Subjects

In- and exclusion criteria have been published previously. In short, patients were eligible to participate if they were between 35 and 80 years old and had CD that had been stable for at least one year on the Tsui scale of dystonia severity with 3-monthly BoNT injections. Exclusion criteria were other neurological conditions at inclusion or in the past, treatment with deep brain stimulation for dystonia, use of medication with a known dopaminergic or serotonergic effect during the study or in the 20 weeks preceding the baseline scans, and pregnancy or lactation. Neurological examination was normal except for dystonia, myoclonus and tremor in all patients. BoNT injections were administered at the first day of the medication trial or a maximum of 7 days prior/after starting the medication. Thus, baseline scans were acquired within a week of BoNT injections and follow-up scans were made between 5-7 weeks after BoNT injections. This study was approved by the Ethical Committee of the AMC, and informed consent was obtained in all patients.

Scoring neurological and psychiatric symptoms

Patients were systematically neurologically and psychiatrically examined as published before. This examination consisted of video recordings, a psychiatric interview, and questionnaires. The detailed results of the effect of escitalopram and placebo on these examinations have been reported previously. Considering the relatively small sample size of the current study, we only incorporated the Clinical Global Impression Scale (CGI), which is a 7-point scale of disease severity, were lower scores indicate less symptoms. This question was answered for jerks, psychiatric symptoms and dystonia separately, both objectively by the physician and subjectively by the patient. We used...
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the CGI to classify patients as responders (defined as a decrease of ≥ 1 point on CGI compared to baseline) or non-responders (CGI same or higher compared to baseline). This was done for the three symptoms separately and by the patient and physician separately.

Experimental design and treatment
Patients in the present imaging sub-study participated in a larger randomized, double-blind, placebo-controlled, crossover trial, containing a total of 53 patients. For the details regarding randomization and treatment assignment we refer to our previously published article. In short, patients were randomly assigned to first receive one of two treatment options for the duration of 6 weeks: escitalopram 10 mg once daily, or placebo. Randomization per block of 4 subjects was performed and the treatment was blinded for both the patients and the investigator. The patients we report on in the current substudy are a subgroup that had baseline SPECT scans before start of the medication trial. Medication was started immediately after all three baseline scans were performed (see below). The repeat SPECT scans, as well as the neurological and psychiatric examinations, were performed after a six week treatment course with escitalopram or placebo. At the same time blood for analysis of plasma levels of escitalopram was withdrawn and stored at -20 °C until analysis. Samples were analysed in batches of 10-20 samples at a time using a validated LC-MS/MS method (range of detection 5-500 µg/L). The last dosage of medication had been less than 24 hours before the scan for both scans. After a washout period of 2-6 weeks, interventions were switched. The halftime of escitalopram is 30 hours, so a minimum period of 2 weeks was sufficient to washout escitalopram completely. Because of radiation burden, scans were not repeated after the second treatment round.

SPECT imaging
All patients received 300 mg potassium iodide to block thyroid uptake of free radioactive iodide before administration of the tracer. Both striatal DAT and midbrain/diencephalon SERT were imaged using [123I]FP-CIT SPECT, a technique that has been validated before. Subjects received a mean dose of 100 MBq of [123I]FP-CIT intravenously (produced according to GMP criteria by GE Healthcare) as a bolus. Scans were performed 2 hours after bolus injection to visualize and quantify the SERT binding in diencephalon/midbrain and 3 hours after bolus injection to visualize and quantify the DAT binding in the striatum. To visualize striatal D2/3 receptor binding, subjects received a 56 MBq bolus of [123I]IBZM intravenously (produced according to GMP criteria by GE Healthcare) followed by continuous infusion of 14 MBq/h of [123I]IBZM until the end of the scan to achieve unchanging regional brain activity levels. Acquisition of the images was
started 2 hours after the bolus injection.25 27 All SPECT studies were performed on a 12-detector single-slice brain-dedicated scanner (Neurofocus 810, which is an upgrade of the Strichmann Medical Equipment) with a full-width at half-maximum resolution of approximately 6.5 mm, throughout the 20-cm field-of-view. After positioning of the subjects with the head parallel to the orbitomeatal line, axial slices parallel and upward from the orbitomeatal line to the vertex were acquired in 5 mm steps. An average of 15 slices was acquired in a 64x64 matrix. Scanning time was 3.5 minutes per slice for $^{[123]}$FP-CIT and 5 minutes per slice for $^{[123]}$IBZM SPECT studies. The energy window was set at 140-178 keV. Images were reconstructed in 3-D mode and analysed blindly by one observer (EZ). To quantify extrastitial SERT, fixed regions of interest (ROIs) for the diencephalon and midbrain combined were positioned as earlier described.22 To quantify striatal DAT, fixed ROIs for the whole striatum were positioned as earlier described.28 The cerebellum was used as reference region both for midbrain/thalamus SERT binding as well as for striatal DAT binding.22 For the $^{[123]}$IBZM images, fixed ROIs for the striatum were positioned and the occipital cortex on the same slices was used as reference region.29 In all cases specific to non-specific binding ratios were calculated as [(activity in ROI – activity in reference region)/activity in reference region], representing the binding potential (BPND).30 BPND is a combined measure of the density of available neuroreceptors/transporters and tracer affinity to the neuroreceptor/transporter. SERT occupancy was calculated as (BPND at baseline - BPND after escitalopram)/BPND at baseline * 100%.

**Statistical analysis**

Mann-Whitney U test was used to calculate differences in BPND between different groups and Wilcoxon matched pairs signed rank sum T test was used to calculate differences in BPND before and after treatment. Spearman’s correlation was used to examine a possible correlation between plasma levels and SERT occupancy. Analyses were carried out using SPSS version 24 and differences were considered significant at p<0.05.

**RESULTS**

In total, in 10 patients treated with escitalopram and in 8 patients treated with placebo before and after treatment SPECT scans were acquired. Despite indicating that they took all medication, two patients in the escitalopram group had non-measurable plasma levels and were excluded from the remainder of the analyses. As expected, none of the patients in the placebo group had measurable plasma levels of escitalopram. Baseline characteristics are depicted in table 1.
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Escitalopram (n=8)</th>
<th>Placebo (n=8)</th>
<th>P-value</th>
</tr>
</thead>
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<tr>
<td>Age, mean (SD)</td>
<td>56.4 (9.1)</td>
<td>56.8 (10.3)</td>
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</tr>
<tr>
<td>Male, n (%)</td>
<td>4 (50%)</td>
<td>6 (75%)</td>
<td>0.61</td>
</tr>
<tr>
<td>Jerks, n (%)</td>
<td>4 (50%)</td>
<td>6 (75%)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

n = number, SD = standard deviation

Difference between baseline scans and scans after treatment

There was no significant difference in extrastriatal SERT, striatal DAT or striatal D2/3R BPND between baseline and treatment with placebo. In patients treated with escitalopram, SERT BPND and DAT BPND were lower and D2/3R BPND was higher after treatment compared to baseline. However, the variability of the measurements was high and results failed to reach statistical significance (table 2).

Table 2. Comparison of SPECT measures (BPND) between pre- and post-treatment. Extrastriatal SERT and striatal DAT were measured with [123I]FP-CIT SPECT, and striatal D2/3R with [123I]IBZM SPECT

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escitalopram (n=8)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SERT</td>
<td>0.18 (0.12-0.31)</td>
<td>0.05 (-0.02)-0.28</td>
<td>0.26</td>
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<tr>
<td>DAT</td>
<td>3.31 (2.47-4.10)</td>
<td>2.90 (2.65-3.97)</td>
<td>0.40</td>
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<tr>
<td>D2/3R</td>
<td>0.71 (0.60-0.92)</td>
<td>0.89 (0.50-0.92)</td>
<td>0.60</td>
</tr>
<tr>
<td>Placebo (n=8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERT</td>
<td>0.28 (0.16-0.38)</td>
<td>0.20 (0.08-0.39)</td>
<td>0.40</td>
</tr>
<tr>
<td>DAT</td>
<td>3.54 (3.40-3.62)</td>
<td>3.71 (3.34-4.56)</td>
<td>0.61</td>
</tr>
<tr>
<td>D2/3R</td>
<td>0.92 (0.62-1.13)</td>
<td>0.88 (0.84-1.03)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Differences between SSRI and placebo

There was a trend towards a lower median diencephalon/midbrain SERT BPND in patients treated with escitalopram (0.05 (IQR -0.02-0.28) compared to patients treated with placebo (0.20 (IQR 0.08-0.39); p=0.13). Median SERT occupancy was 64.6% in the escitalopram group (IQR 1.2-91.9%). There was no significant difference in DAT BPND (p=0.40) or D2/3R BPND (p=0.46) between the treatment groups.
After treatment with escitalopram, patients that indicated on the CGI that dystonia improved (n=6) had a strong trend towards lower extrastriatal SERT BP\textsubscript{ND} on the post-treatment scan compared to patients (n=2) that indicated that severity of dystonia was the same or worse (0.03 (IQR -0.04-0.10) vs. 0.33 (IQR 0.29-NA); p=0.07). The median occupancy of SERT was 71.6% in patients that indicated dystonia improved on the CGI, but the SERT was not occupied in patients that did not indicated improvement (p=0.14).

Patients (n=5) that indicated on the CGI that their psychiatric symptoms (depressive symptoms and anxiety) improved after treatment with escitalopram had significantly lower SERT BP\textsubscript{ND} on the post-treatment scan compared to patients (n=3) that indicated that severity of psychiatric symptoms was the same or worse (0.01 (IQR -0.04-0.05) vs. 0.29 (IQR 0.25-NA); p=0.04). The median occupancy of SERT was 76.5% in patients that indicated improvement of psychiatric symptoms, but SERT was not occupied in patients that did not indicate improvement (p=0.04).

In the escitalopram group there was no significant difference in extrastriatal SERT BP\textsubscript{ND} or occupancy between patients who indicated they did or did not improve on jerks. There was no difference in DAT BP\textsubscript{ND} and D2/3R BP\textsubscript{ND} between escitalopram treated patients who indicated they improved on dystonia, jerks or psychiatric symptoms compared to patients who did not improve. There was also no difference in SERT, DAT and D2/3R BP\textsubscript{ND} between escitalopram treated patients who did and did not improve on dystonia, jerks or psychiatric symptoms according to the physicians.

Median plasma level of escitalopram was 21.0 µg/L (IQR 10.9-25.8). There was no correlation between SERT occupancy and plasma levels of escitalopram (correlation coefficient 0.21, p=0.61). There was also no correlation between SERT BP\textsubscript{ND} either pre- or posttreatment and plasma levels of escitalopram.

In the placebo group there were no differences in SERT, DAT and D2/3R BP\textsubscript{ND} between patients who did and did not improve on dystonia, jerks or psychiatric symptoms according to either the patients or the physicians.

**DISCUSSION**

In our study we found a strong trend towards a lower midbrain/diencephalon SERT BP\textsubscript{ND} in CD patients treated with escitalopram compared to placebo. The lower SERT BP\textsubscript{ND} in the escitalopram group represents occupancy of SERT of 65%. The lack of a statistical significant occupancy of SERT after a 6 week treatment course was likely due
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to a combination of factors. One factor was low baseline extrastriatal SERT BP\textsubscript{ND}. Low baseline SERT binding, is in line with previous human \textsuperscript{[123]}I-FP-CIT SPECT studies\textsuperscript{22,31,32}, and is related to the modest affinity of the radiotracer for the SERT.\textsuperscript{33} The BP\textsubscript{ND} levels did reduce to around 0 after treatment (see Table 2), reflecting substantial SERT occupancy, but the absolute difference in BP\textsubscript{ND} was small. Another factor was that in our study the last tablet was taken the day before the scans were made. In healthy subjects using 10 mg escitalopram once daily for 10 days, SERT occupancy in the midbrain 6 and 54 hours after ingestion of the last tablet showed a decrease of 82\% after 6 hours to 63\% after 54 hours.\textsuperscript{14} It is likely this effect also contributed to the relatively low SERT occupancy we found. However, our present study was not designed to test whether \textsuperscript{[123]}I-FP-CIT SPECT can be used to measure SERT occupancy by an SSRI, as this has been proven in several other experimental studies\textsuperscript{12,31}, but just to evaluate the relationship between SERT occupancy and clinical effects.

In the present study, there was no significant relationship between SERT occupancy and plasma levels of escitalopram. Previous molecular imaging studies have also reported the absence of a relationship between SERT occupancy and plasma levels, likely due to the ceiling effect where maximum SERT occupancy of around 80\% is reached at relatively low dosages of SSRIs.\textsuperscript{13,31} As expected, in patients treated with placebo there were no significant differences in SERT, DAT or D2/3R BP\textsubscript{ND}.

The most surprising, but interesting, finding of our study was that after escitalopram treatment patients with high SERT occupancy reported a better clinical effect on dystonia and psychiatric symptoms compared to patients with no SERT occupancy. This effect was defined as an increase on self-reported CGI severity scores and there was no difference in an objective physician CGI score of disease severity. Because of the small sample size we did not incorporate scores from depression or anxiety questionnaires, such as the Beck Depression Inventory or Beck Anxiety Inventory. However, our finding indicates that patients in whom most SERTs are blocked by escitalopram experience a larger subjective treatment effect. Such a relationship between SERT occupancy and treatment effect has not been demonstrated in patients with major depression.\textsuperscript{16} However, in patients with obsessive-compulsive disorder using sertraline, another SSRI, a positive relationship was detected between pre-treatment/baseline SERT BP\textsubscript{ND} measured with \textsuperscript{[123]}I-β-CIT SPECT in the diencephalon, as well as higher SERT occupancy after treatment, and better treatment response.\textsuperscript{34} This is of interest, since sertraline is the SSRI with the highest affinity for the DAT, thus an effect of SERT occupancy on treatment response can be debated. An alternative explanation for our finding of higher levels of SERT occupancy in patients with a positive subjective response is that increased SERT occupancy led patients to give more positive answers. This was previously shown.
in patients with major depression and severe pessimistic attitudes who had low extracellular serotonin levels. These levels could be raised by the serotonin releaser d-fenfluramine, which led to more optimism in these patients.\textsuperscript{35} A pessimistic attitude at baseline could have led to a low score given by the patients and a shift towards optimism would have made the patients give themselves a better score. This might explain why in our study there was a relationship between SERT occupancy and the positive subjective scores of the patients, but the lack of such a relationship between SERT occupancy and the objective physician scores.

In contrast with our hypothesis, no significant differences were found in striatal DAT $BP_{ND}$ after treatment with escitalopram. Albeit not significant, the median DAT $BP_{ND}$ we found in this current study was 14\% lower after treatment with escitalopram compared to baseline. From studies in patients with major depression we know that SSRIs also have an effect on the dopaminergic system, but the decrease in DAT $BP_{ND}$ is much smaller (around 20\%) than the decrease in SERT $BP_{ND}$ (40-70\%). Furthermore, the decrease in DAT $BP_{ND}$ is smaller in older patients, independently from SERT $BP_{ND}$ and the patients in our study are slightly older than in the described study (56 vs 42 years).\textsuperscript{11} There are also indications that the decrease in DAT $BP_{ND}$ is related to the used reference region, at least when using non-selective tracers like $[^{123}]$FP-CIT. When using the occipital cortex as reference tissue, a larger effect on DAT $BP_{ND}$ was found than when using the cerebellum, probably because of a higher concentration of SERTs in the occipital cortex. Therefore, in this study we used the cerebellum to assess the non-specific binding. Other factors that can explain why some studies found an effect of SSRIs on striatal DAT $BP_{ND}$ while others did not, are intravenously given medication versus oral ingestion, chronic treatment with SSRIs versus acute administration of a single dose, and maybe the cohort under study (patients like CD versus healthy controls may show a different neurotransmitter response).\textsuperscript{31} At baseline in our previous study in the same patient group, we found a positive correlation between baseline DAT $BP_{ND}$ and severity of myoclonus measured with the UMRS.\textsuperscript{7} There was no clinical effect of escitalopram on the severity of myoclonus, in line with the absence of a difference in DAT $BP_{ND}$ in the treatment group.

We also did not find significant changes in D2/3R $BP_{ND}$ after treatment with escitalopram. In healthy controls significantly decreased $[^{11}C]$raclopride binding to striatal D2 receptors and increased plasma metabolites of dopamine were found after a single dose of 60 mg d-fenfluramine implicating serotonergic modulation of synaptic concentrations of dopamine.\textsuperscript{36} In patients with major depression, overall no treatment effect on D2/3R $BP_{ND}$ was found using $[^{123}]$IBZM SPECT, but there were indications of a relationship between D2/3R $BP_{ND}$ before and after treatment with either the SSRI paroxetine or fluoxetine. Patients that responded well to SSRIs had lower D2/3R $BP_{ND}$ at baseline and
D2/3R $B_{\text{ND}}$ increased after treatment. Patients that did not respond to treatment with SSRIs had higher D2/3R $B_{\text{ND}}$ at baseline and D2/3R $B_{\text{ND}}$ decreased after treatment. We did not find a correlation between D2/3R $B_{\text{ND}}$ and treatment response, neither for the objective scores of the physicians nor for the subjective scores from the patients. $[^{123}\text{I}]\text{IBZM SPECT}$ is a tracer that binds to both the dopamine D2 as well as D3 receptor, with D2 receptors outnumbering D3 receptors in the striatum. D3 receptors are hypothesized to be involved in the inhibition of spontaneous motor activity and in the reward system. D3 receptor mRNA has been shown to be present in striatal neurons that project to the globus pallidus pars interna (GPi). D3 receptor upregulation would lead to over-inhibition of GPi target areas. In patients with Parkinson’s disease low firing rate of the GPi is linked to the occurrence of levo-dopa induced dyskinesias. A similar situation could be a pathophysiological mechanism in dystonia, especially since deep brain stimulation of the GPi is effective in decreasing motor symptoms. With a non-selective D2/3R tracer a selective increase of D3R could be missed, especially in a study with a small sample size, like ours. It would be interesting to perform a similar study with serial scans using the PET tracer $[^{11}\text{C}]\text{PHNO}$, which has a D3 selectivity that is 20 times higher than D2 in patients with CD. This study has some limitations. The most important limitation is the sample size. This is especially important when measuring extrastriatal SERT $B_{\text{ND}}$ with $[^{123}\text{I}]\text{FP-CIT SPECT}$ of which it has been established that the $B_{\text{ND}}$ is lower with a larger range compared to striatal DAT $B_{\text{ND}}$ (measured with $[^{123}\text{I}]\text{FP-CIT SPECT}$) or D2/3R $B_{\text{ND}}$ (measured with $[^{123}\text{I}]\text{IBZM SPECT}$). A higher range in measurements makes it statistically more difficult to detect small differences. Furthermore, we used $[^{123}\text{I}]\text{FP-CIT}$ to image both DAT and SERT $B_{\text{ND}}$ in the same patients. $[^{123}\text{I}]\text{FP-CIT}$ is a non-selective DAT/SERT SPECT tracer that can reliably be used to measure SERT $B_{\text{ND}}$ in the diencephalon/midbrain but not in other relevant brain regions like the striatum. In addition, we had to exclude two patients from the escitalopram group because of non-detectable escitalopram plasma levels. This might indicate they did not take medication, however we cannot exclude that they were so-called fast metabolizers. In a study where 8 healthy controls were treated with the SSRI paroxetine for 8 days, and all tablets were ingested under supervision, one patient had non-detectable plasma levels of paroxetine and was considered a fast metabolizer. We could measure plasma levels of $\geq 5 \mu g/L$ and a plasma level of $5 \mu g/L$ has been proven to give an occupancy of $>80\%$ in the midbrain. However, SERT occupancy in these particularly two patients was in the negative range ($B_{\text{ND}}$ was even somewhat higher after treatment compared to baseline; data not shown) making it unlikely that these patients had plasma levels just below the detection range and making it more likely that they did not take study medication. Lastly, here we only measure short term effects with a treatment duration of 6 weeks. It is very well possible
that with a longer treatment duration neuroadaptive changes will occur and results will be different. It would be interesting to investigate this in a larger study with a longer treatment duration, for example a prospective cohort of patients with CD that are started on an SSRI to reduce psychiatric symptoms and undergo SPECT scans before treatment and after 3-6 months of treatment.

In conclusion, we performed the first serial SPECT study in patients with CD treated with escitalopram. We found a trend towards lower SERT $B_{PD}$ and consequently higher SERT occupancy after treatment with escitalopram, but more interesting we found that within the group treated with escitalopram patients who reported subjective improvement in dystonia and/or psychiatric symptoms had significantly lower SERT $B_{PD}$ and higher SERT occupancy than patients who did not improve.

**Acknowledgements**

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**Conflicts of interest**

Escitalopram and placebo were a gift from Lundbeck Pharmaceutical Company. Lundbeck played no role in the trial design, data analysis or writing of the article.
REFERENCES


Chapter 6


