Comparison of the in-vivo muscarinic cholinergic receptor availability in patients treated with clozapine and olanzapine

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Abstract
Clozapine and olanzapine are two atypical antipsychotics that bind to a broad range of receptors in vitro. Our group previously reported on the binding of clozapine and olanzapine to muscarinic receptors in vivo. Based on these data, a direct comparison of the muscarinic receptor availability in vivo under treatment with these atypical antipsychotics was performed. $^{[123]}$I IQNB SPECT scans were obtained in seven subjects treated with a high dose (20 mg) of olanzapine and seven subjects treated with a moderate dose (mean 275.0 mg, range 200–450 mg) of clozapine. Muscarinic receptor-binding indices were determined for basal ganglia, cortex, thalamus and pons. When comparing moderate-dose clozapine with high-dose olanzapine, significantly lower muscarinic receptor availability was found for clozapine in all four cortical regions of interest. Our results suggest that treatment with clozapine results in a stronger blockade of the muscarinic cholinergic receptors than with olanzapine. These results are compatible with the higher rates of anticholinergic side-effects seen with clozapine in clinical practice.

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Key words: Clozapine, IQNB, olanzapine, muscarinic receptors, schizophrenia, SPECT.

Introduction
Clozapine, a tetracyclic dibenzodiazepine, is still considered to be the prototype of atypical antipsychotics as it combines superior clinical efficacy, particularly in treatment-refractory schizophrenic patients, with a lack of extrapyramidal side-effects (Kane et al., 1988; Rosenheck et al., 1997). However, the use of clozapine is complicated by serious and potentially fatal side-effects, which led to a search for novel antipsychotics with excellent efficacy and better tolerability. Olanzapine, a thienobenzodiazepine, is an atypical antipsychotic that is structurally related to clozapine. In clinical studies olanzapine has proven effective for the treatment of positive and negative symptoms of schizophrenia (Beasley et al., 1996, 1997; Tollefson et al., 1997a) with relatively few side-effects and minimal extrapyramidal symptoms (Tollefson et al., 1997b; Tran et al., 1997). In direct comparison of the clinical efficacy of both drugs, some studies showed equal efficacy for clozapine and olanzapine (Littrell et al., 2000; Tollefson et al., 2001; Volavka et al., 2002), while clozapine has proven more effective in other studies (Conley et al., 1999).

In-vitro studies of the receptor-binding profile show that clozapine has a high affinity for a large variety of neurotransmitter receptors, including 5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_{5}$, 5-HT$_{6}$, D$_{1}$ and D$_{2}$ receptors, but has only moderate affinity at dopamine D$_{3}$ receptors (Bymaster et al., 1996; Schotte et al., 1996). Olanzapine binds with high affinity to dopamine, serotonin, histamine and norepinephrine receptors (Bymaster et al., 1999). Both clozapine and olanzapine bind with high affinity to all five subtypes of the muscarinic receptor (Bolden et al., 1992; Bymaster et al., 1996).

$^{[123]}$I IQNB (quinuclidinyl benzilate) SPECT was previously used to demonstrate that treatment with olanzapine results in a dose-dependent, mild-to-moderate muscarinic receptor occupancy (Raedler et al., 2000), while treatment with clozapine resulted in a dose-dependent, moderate-to-high muscarinic receptor occupancy (Raedler et al., 2003). In order to better characterize the binding properties of both
antipsychotics in vivo, this paper presents a direct comparison of the binding of clozapine and olanzapine to the muscarinic cholinergic receptors in vivo.

Methods

Subjects

As previously reported (Raedler et al., 2000, 2003) 14 schizophrenic in-patients (12 males, 2 females; mean age 38.8 ± 10.0 yr; illness duration 17.1 ± 8.7 yr) were recruited from the in-patient service of the National Institute of Mental Health (NIMH), Bethesda, MD, USA (for demographic information see Table 1). Before inclusion in the study, each subject gave written informed consent to participate in a [123]I]IQNB SPECT study according to a protocol approved by the Institutional Review Board and the Radiation Safety Committee of NIMH.

All subjects received a thorough medical, neurological and psychiatric evaluation prior to enrolment in the study and were free of active medical problems. The clinical work-up included a brain MRI to rule out structural lesions as well as for co-registration with the SPECT scans. All subjects were chronically ill and were diagnosed with schizophrenia (n = 12) or schizoaffective disorder (n = 2) according to DSM-IV criteria.

All patients had been treated with different typical and atypical antipsychotics prior to the beginning of the study. Antipsychotics and anticholinergic drugs were tapered over a 2-wk period before the start of the study. Concomitant medications were held constant in dosage for at least 2 wk prior to SPECT scanning. The treatment schedules are explained in detail as they differed for subjects treated with clozapine and olanzapine.

Clozapine sample

[123]I]IQNB SPECT scans were obtained in seven subjects treated with clozapine. Following a gradual titration, the dose of clozapine was optimized based on clinical considerations. IQNB SPECT scans were acquired after a minimum of 2 wk treatment with a moderate dose of clozapine (mean 275.0 mg/d). Plasma levels for clozapine ranged from 150 ng/ml to 396 ng/ml.

Olanzapine sample

Treatment with olanzapine was administered in a fixed-dose fashion. Seven subjects were studied with [123]I]IQNB SPECT after at least 2 wk treatment with a high dose (20 mg/d) of olanzapine.

Clinical and neurological ratings

Clinical and neurological ratings were obtained on all schizophrenic subjects on the day of the [123]I]IQNB SPECT scan. Clinical ratings consisted of the Positive and Negative Symptoms Scale

Table 1. Demographic information of the subjects treated with clozapine (n = 7) and olanzapine (n = 7)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (yr)</th>
<th>Diagnosis</th>
<th>Illness duration</th>
<th>Dosage (mg/d)</th>
<th>PANSS total</th>
<th>Prior antipsychotic</th>
<th>Prior anticholinergic</th>
<th>Concomitant medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 34</td>
<td>SA</td>
<td>13</td>
<td>225</td>
<td>36</td>
<td>Risperidone</td>
<td>Benztropine</td>
<td>Valproic acid</td>
<td></td>
</tr>
<tr>
<td>M 44</td>
<td>SA</td>
<td>29</td>
<td>200</td>
<td>81</td>
<td>Haloperidol</td>
<td>Benztropine</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>M 38</td>
<td>CUS</td>
<td>18</td>
<td>300</td>
<td>91</td>
<td>Thioridazine</td>
<td>Benztropine</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>M 46</td>
<td>CPS</td>
<td>20</td>
<td>200</td>
<td>71</td>
<td>Molindone</td>
<td>Benztropine</td>
<td>Ranitidine</td>
<td></td>
</tr>
<tr>
<td>M 26</td>
<td>CUS</td>
<td>4</td>
<td>450</td>
<td>66</td>
<td>Risperidone</td>
<td>Trihexyphenidyl</td>
<td>Propranolol</td>
<td></td>
</tr>
<tr>
<td>M 38</td>
<td>CPS</td>
<td>16</td>
<td>250</td>
<td>51</td>
<td>Fluphenazine</td>
<td>Benztropine</td>
<td>Ranitidine</td>
<td></td>
</tr>
<tr>
<td>M 48</td>
<td>CPS</td>
<td>21</td>
<td>300</td>
<td>95</td>
<td>Haloperidol</td>
<td>Benztropine</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 44</td>
<td>CPS</td>
<td>17</td>
<td>20</td>
<td>52</td>
<td>Risperidone</td>
<td>Benztropine</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>F 56</td>
<td>CPS</td>
<td>30</td>
<td>20</td>
<td>67</td>
<td>Thioridazine</td>
<td>Trihexyphenidyl</td>
<td>Ibuprofen prn</td>
<td></td>
</tr>
<tr>
<td>M 41</td>
<td>CUS</td>
<td>20</td>
<td>20</td>
<td>99</td>
<td>Haloperidol</td>
<td>Benztropine</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>M 24</td>
<td>CPS</td>
<td>6</td>
<td>20</td>
<td>74</td>
<td>Haloperidol</td>
<td>Benztropine</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>M 28</td>
<td>CUS</td>
<td>9</td>
<td>20</td>
<td>56</td>
<td>Thiothixene</td>
<td>Benztropine</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>M 50</td>
<td>CUS</td>
<td>30</td>
<td>20</td>
<td>68</td>
<td>Risperidone</td>
<td>Trihexyphenidyl</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>F 26</td>
<td>CPS</td>
<td>7</td>
<td>20</td>
<td>70</td>
<td>Fluphenazine</td>
<td>Benztropine</td>
<td>Atenolol</td>
<td></td>
</tr>
</tbody>
</table>

PANSS, Positive and Negative Symptoms Scale; SA, schizoaffective disorder; CUS, chronic undifferentiated schizophrenia; CPS, chronic paranoid schizophrenia.
(PANSS). Extrapyramidal signs, parkinsonism and dyskinesia were assessed with the Modified Abnormal Involuntary Movement Scale (AIMS).

**[123]I[123]I]IQNB SPECT procedure and image analysis**

The SPECT procedure and the image analysis followed previously described principles (Norbury et al., 2004; Raedler et al., 2000). All [123]I[123]I]IQNB SPECT scans were obtained under identical conditions. All subjects received a non-radioactive iodine solution to minimize thyroid uptake of radioactive iodine. Each subject was given an intravenous injection of ~7 mCi (mean 7.3 mCi, range 4.0–10.0 mCi) of isomerically pure [R,S]-[123]I[123]I]IQNB at approximately 13:00 hours and subjects returned to the SPECT laboratory for a 60-min SPECT scan 21 h after the injection. During imaging, subjects reclined comfortably in the chair of a Ceraspect camera (Digital Scintigraphics, Waltham, MA, USA). SPECT data were acquired with a high-resolution collimator (7.5 mm FWHM) in a 120-projection step-and-shoot mode.

An MRI scan was obtained on each subject with a 1.5 T Signa scanner (General Electric Medical Systems, Milwaukee, WI, USA). Based on gross anatomical features, the SPECT images were aligned to the MRI scans. Anatomical regions of interest (ROIs) for cerebellum, pons, thalamus, caudate and putamen as well as medial-frontal, medial-lateral, temporal and occipital cortex were drawn on five contiguous slices of the MRI scan to form a volume of interest (VOI) for each area. These ROIs were then superimposed onto the corresponding slices of the co-registered SPECT scans. Data from right- and left-sided structures were averaged together. The average concentration of activity in each VOI was determined by dividing the counts per minute by the volume of the VOI; these data were decay-corrected and calibrated to nCi/ml tissue using data from scans of the flood phantom. Finally, these data were normalized to the injected dose to yield values in units of nCi/ml tissue per mCi injected dose and cerebellum data, assumed to represent non-specific background activity, was subtracted from each VOI value.

**Statistical analysis**

Statistical analysis of the data was performed with STATISTICA (StatSoft, Inc., Tulsa, OK, USA) and Microsoft Excel (Microsoft Corp., Redmond, WA, USA). The Student’s t test for independent samples was applied to compare data from schizophrenic subjects treated with clozapine and olanzapine. The \( \chi^2 \) test was used for the analysis of 2 x 2 frequency tables.

### Table 2. Muscarinic receptor availability in nCi/ml tissue per mCi injected (mean ± s.d.) under treatment with moderate-dose clozapine (275.0 ± 87.8 mg/d) vs. high-dose olanzapine (20 mg/d)

<table>
<thead>
<tr>
<th>Volume of interest</th>
<th>Clozapine ((n = 7))</th>
<th>Olanzapine ((n = 7))</th>
<th>( t )</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial frontal cortex</td>
<td>27.9 ± 11.1</td>
<td>41.4 ± 5.7</td>
<td>-2.84</td>
<td>0.01</td>
</tr>
<tr>
<td>Lateral frontal cortex</td>
<td>27.3 ± 11.2</td>
<td>41.4 ± 4.4</td>
<td>-3.09</td>
<td>0.009</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>34.3 ± 12.4</td>
<td>51.2 ± 5.8</td>
<td>-3.25</td>
<td>0.007</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>31.7 ± 12.7</td>
<td>45.8 ± 5.5</td>
<td>-2.69</td>
<td>0.02</td>
</tr>
<tr>
<td>Caudate</td>
<td>31.0 ± 12.1</td>
<td>38.9 ± 6.0</td>
<td>-1.56</td>
<td>0.15</td>
</tr>
<tr>
<td>Putamen</td>
<td>33.7 ± 13.6</td>
<td>46.7 ± 9.5</td>
<td>-2.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Thalamus</td>
<td>6.5 ± 3.0</td>
<td>9.9 ± 3.9</td>
<td>-1.86</td>
<td>0.09</td>
</tr>
<tr>
<td>Pons</td>
<td>4.5 ± 3.4</td>
<td>2.7 ± 2.6</td>
<td>1.12</td>
<td>0.28</td>
</tr>
</tbody>
</table>

**Results**

The two samples of subjects treated with clozapine \((n = 7)\) and olanzapine \((n = 7)\) were well matched for age \((39.1 ± 7.6\text{ yr} vs. 38.4 ± 12.6\text{ yr}; \text{t} = 0.12, \text{d.f.} = 12, \text{p} < 0.91)\), illness duration \((17.3 ± 7.7\text{ yr} vs. 17.0 ± 10.3\text{ yr}; \text{t} = 0.06, \text{d.f.} = 12, \text{p} < 0.95)\), gender distribution \((\chi^2 = 2.33, \text{d.f.} = 1, \text{p} = 0.13)\), PANSS total score \((70.1 ± 21.3\text{ vs. 69.4 ± 15.2}; \text{t} = 0.07, \text{d.f.} = 12, \text{p} < 0.95)\), PANSS subscores or AIMS score \((5.3 ± 4.0\text{ vs. 6.6 ± 6.0}; \text{t} = -0.47, \text{d.f.} = 12, \text{p} < 0.65)\).

**Moderate-dose clozapine (275.0 ± 87.8 mg/d) vs. high-dose olanzapine (20 mg/d)**

Comparing the [123]I[123]I]IQNB binding in subjects treated with moderate doses of clozapine vs. high dose olanzapine, the muscarinic receptor availability was lower in all VOIs except for pons in subjects treated with clozapine. These differences reached statistical significance in all four cortical ROIs (see Table 2).

**Discussion**

In-vivo and ex-vivo studies of medication effects on receptor binding can lead to differing results (Kapur et al., 2001). In-vitro studies of the binding properties can have potential methodological problems including that they are rarely performed in human tissue and are susceptible to exogenous factors (e.g. the medium used). In addition, in-vitro studies assess only the effects of a mother substance and not of active metabolites. PET and SPECT studies share the advantage that they allow the assessment of medication effects directly in the living human brain and can thus
expand our knowledge about in-vivo effects of psychotropics.

In this study [123I]IQNB SPECT was used to measure the binding of clozapine and olanzapine to central muscarinic acetylcholine receptors in vivo. Comparing moderate-dose clozapine with high-dose olanzapine, treatment with clozapine resulted in lower muscarinic receptor availability in all VOIs. These differences reached statistical significance in all four cortical VOIs.

Our results suggest that in-vivo treatment with clozapine results in lower muscarinic cholinergic receptor availability and thus higher muscarinic receptor occupancy than treatment with clinically comparable doses of olanzapine.

These results are compatible with the few studies that have focused on the in-vivo effects of antipsychotics on muscarinic receptors. In the only other SPECT study published, [123I]IDEX was used as a SPECT tracer to compare the muscarinic receptor binding under treatment with olanzapine and risperidone. Compared with unmedicated healthy controls, subjects on olanzapine showed significantly lower muscarinic receptor availability in the striatum and cortex, while treatment with risperidone resulted in slightly lower muscarinic receptor availability (Lavalaye et al., 2001).

Serum anticholinergic levels were used as another measure of anticholinergic effects in vivo. Treatment with olanzapine led to anticholinergic levels that were about one fifth of those found in patients treated with clozapine, indicating stronger anticholinergic activity for clozapine. At the same time anticholinergic side-effects were more frequent and more severe under clozapine (Chengappa et al., 2000). In patients with dementia, olanzapine but not risperidone led to a significant increase in serum anticholinergic levels (Mulsant et al., 2004).

The in-vitro binding properties to muscarinic cholinergic receptors have been evaluated for both clozapine and olanzapine. Using rat tissue and cell lines transfected with muscarinic receptors, the affinity of both olanzapine and clozapine to the different muscarinic receptor subtypes were in the nanomolar range (Bymaster et al., 1996, 1999). However, when using a different medium for the assays, the affinity of both olanzapine and clozapine to the muscarinic receptor was found to be substantially weaker (Bymaster and Falcone, 2000; Zhang and Bymaster, 1999).

Some of the potential side-effects of both clozapine and olanzapine, such as impaired accommodation, dry mouth, urinary retention and constipation, have been linked to antagonistic effects of both drugs on the muscarinic receptors. At the same time, anticholinergic drugs are used to treat unwanted motor side-effects of antipsychotics, which result from a blockade of striatal dopamine D_{2} receptors.

Clozapine is virtually free of motor side-effects while olanzapine has a reduced risk of motor side-effects (Tollefson et al., 1997b). The demonstrated effect of both clozapine and olanzapine on the availability of the muscarinic receptors in vivo may account for some of the side-effects as well as the reduced risk of extrapyramidal motor side-effects associated with both antipsychotics.

Beyond the effects of extrapyramidal motor side-effects, muscarinic antagonism has been viewed as a liability due to its presumed potential to cause cognitive impairment. Clinical observations and pre-clinical studies have challenged the concept that clozapine acts as a pure muscarinic antagonist. While clozapine initially causes dry mouth, treatment with clozapine can lead to increased salivation that can be treated with anticholinergic agents such as pirenzipine. In-vitro studies suggest that clozapine may act in a dose-dependent fashion as a partial agonist at different muscarinic receptor subtypes (Michal et al., 1999; Olianas et al., 1999; Zeng et al., 1997; Zorn et al., 1994). N-desmethylclozapine, the active metabolite of clozapine, is an agonist at the M_{1} receptor (Davies et al., 2005; Sur et al., 2003; Weiner et al., 2004), which may account for some of these effects. Pharmacological manipulation of the muscarinic receptor may have benefits that go beyond prophylaxis and treatment of motor side-effects associated with antipsychotics.

Several limitations should be noted when interpreting the results of our study. IQNB allows the assessment of muscarinic receptor availability but does not distinguish between agonist and antagonist effects of medications (e.g., agonist and antagonist effects of clozapine/N-desmethylclozapine vs. antagonist effects of olanzapine). The sample size of the different groups, in particular the low-dose clozapine group, was small. While a relatively high dose of olanzapine was given, subjects received only moderate doses of clozapine. It is possible that the differences between clozapine and olanzapine might be even larger if higher doses of clozapine were applied. Additional effects of changes in cerebral blood flow or endogenous acetylcholine levels may also affect the results of the SPECT scans. However, both clozapine (Molina Rodriguez et al., 1996) and olanzapine (Gonul et al., 2003) are free of major effects on cerebral blood flow. The results are, therefore, primarily ascribed to direct effects of both medications on the muscarinic receptors.

Further imaging studies of the muscarinic receptor might help to clarify the relationship between...
muscarinic receptor occupancy and anticholinergic side-effects or, possibly, therapeutic effects. Studies with subtype-specific ligands for the muscarinic system might also lead to a better understanding of the role of the different muscarinic subtypes in the pathophysiology and treatment of different psychiatric disorders.

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None.

Statement of Interest

Dr Raedler has participated in scientific meetings organized by Novartis (no honorarium) and has received travel support from Eli Lilly (no honorarium).

References


Davies MA, Compton-Toth BA, Hufeisen SJ, Meltzer HY, Roth BL (2005). The highly efficacious actions of N-desmethylclozapine at muscarinic receptors are unique and not a common property of either typical or atypical antipsychotic drugs: is M1 agonism a pre-requisite for mimicking clozapine’s actions? Psychopharmacology 178, 451–460.


