Relation of Serum Anticholinergicity to Cognitive Status in Schizophrenia Patients Taking Clozapine or Risperidone

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Background: A potential beneficial outcome of treatment with certain of the atypical neuroleptics is the reduced risk of cognitive impairment, stemming from purported low affinity for cholinergic receptors. In vitro experiments have shown that clozapine is highly anticholinergic and risperidone is minimally so. In vivo tests of the anticholinergic burden imposed by these medications and its potential cognitive consequences are needed. This study examines anticholinergic burden in schizophrenia patients taking clozapine and risperidone and tests whether this burden is associated with cognitive deficits.

Method: Serum anticholinergic levels were determined in a sample of 22 chronic schizophrenia patients using the radioreceptor assay method of Tune and Coyle (1980). Fifteen patients received clozapine; 7 received risperidone. Mean ± SD age of the sample, comprising 12 men and 10 women (68% white), was 44.7 ± 8.4 years. Mean ± SD age at onset of schizophrenia illness was 23.5 ± 7.4 years. Two anticholinergic assays based on blood samples collected 1 week apart were available on each patient.

Results: Data indicated that clozapine patients had significantly ($p < .001$) higher anticholinergic levels at both collection points, and levels for both drugs remained stable over time. The clozapine and risperidone patients had essentially equivalent scores on the cognitive measure.

Conclusion: These data suggest that anticholinergicity distinguishes clozapine and risperidone in vivo but that this effect is not associated with differences in global cognitive functioning. Results suggest that clozapine, despite producing moderately high in vivo serum anticholinergic levels, still holds clinical advantage over standard neuroleptics in terms of cognitive side effects. Reasons for this lowered risk of cognitive impairment are discussed.

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The new atypical neuroleptics such as clozapine and risperidone have brought with them increased hope of relief from both the positive and negative symptoms of psychosis and greatly reduced risk of extrapyramidal symptoms and tardive dyskinesia. A less emphasized but equally plausible beneficial outcome of treatment with certain of the atypical neuroleptics is the reduced risk of cognitive impairment, stemming from purported low affinity for cholinergic receptors. In vitro pharmacologic data clearly lead to the expectation among researchers and clinicians that the anticholinergic properties of the atypical neuroleptics would vary. Clozapine is regarded as highly anticholinergic, even more so than standard neuroleptics,[1–3] and risperidone is regarded as minimally anticholinergic.[2,4] Studies have verified the in vivo effects of standard neuroleptics. For instance, Tune and Coyle[5] studied a sample of 76 psychiatric patients medicated with standard neuroleptics such as haloperidol and an adjunctive anticholinergic medication. They found that serum anticholinergic levels were consistently in the range of 10 picomoles per milliliter (pmol/mL) atropine equivalents. In contrast, in vivo studies verifying the anticholinergic activity of the atypical neuroleptics in clinical patients have been lacking. Prior work examining the relation between atypical neuroleptics such as clozapine and cognition in schizophrenia have not included serum assays of anticholinergicity and have only examined relationships to clozapine dose.[6] Such work suggests that clozapine may

induce cognitive deficits particularly in areas such as memory.[6]

This study had 2 goals. First, to determine the anticholinergic burden imposed by clozapine and risperidone in vivo, using plasma assays from a sample of chronic schizophrenia patients. Second, to determine whether the anticholinergic burden of these drugs produced different cognitive effects. To accomplish the latter goal, patients taking clozapine and risperidone were compared on a measure of general cognitive functioning, i.e., the Mini-Mental Status Examination (MMSE). An association between high anticholinergic levels and cognitive impairment was hypothesized, as such a relation has been observed in several other populations (surgical patients and the elderly).[7–9] The study has the advantage of verified DSM-IV diagnoses (Structured Clinical Interview for the DSM-IV[10]) and a single psychotropic drug regimen, i.e., no other anticholinergic drugs were present. Also, patients were assayed twice for serum anticholinergic levels, allowing us to address the issue of stability in anticholinergic drug levels.

METHOD

Subjects

All subjects (N = 22) were patients at Norristown State Hospital (Norristown, Pa.) with a diagnosis of chronic schizophrenia (any subtype) confirmed through the Structured Interview for the DSM-IV.[10] All subjects were free of neurologic or substance use disorders (past year) and other central nervous system problems, as determined by a screening interview and records review. The mean ± SD age of the sample, comprised of 12 men and 10 women, was 44.7 ± 8.4 years (range, 31–58 years), and a total of 68.2% (15/22) was white. The mean ± SD age at onset of schizophrenia was 23.5 ± 7.4 years. The Mini-Mental State Examination[11] was used to assess for general cognitive impairment. A score of below 24 is generally considered to reflect impairment.[12] The Brief Psychiatric Rating Scale was used to assess general psychiatric state. The mean ± SD score on this measure (38.8 ± 8.2, N = 19) suggested that the sample was in a nonacute state (BPRS-Anchored).[13]

Fifteen patients were taking clozapine, and 7 were taking risperidone. Mean ± SD oral clozapine dose was 489.3 ± 190.5 mg/day (range, 200–800 mg/day), with mean ± SD chlorpromazine equivalency value of 333.9 ± 127.6 mg/day (range, 134–536 mg/day). Mean ± SD risperidone oral dose was 4.7 ± 2.1 mg/day (range, 1–7 mg/day). All patients had been taking a stable dose of their single-neuroleptic drug regimen for at least 30 days prior to the study and were not currently taking any other potentially anticholinergic agent or medication that could affect cognition (e.g., benzodiazepines). Blood samples were taken twice, separated by a 1-week interval (Time 1, T1, and Time 2, T2), and all dosages remained stable during this period. The clozapine and risperidone patient groups did not statistically differ in terms of age, gender or race composition, or age at onset of schizophrenia. All subjects provided written informed consent for the blood sample collection as part of a larger study.

Procedure

Blood sample collection procedures were identical at T1 and T2. Blood samples were collected between 8 a.m. and 9 a.m., after breakfast and the morning medication dose. Blood was collected in untreated tubes and clotted at room temperature for 30 minutes, then centrifuged at 2400 g for 10 minutes. Serum was then removed and frozen at –20°C for approximately 6 months. At T1, 1 patient was not available for blood collection, yielding a total of 21 assays for T1, and 22 for T2. The MMSE was administered a few days prior to the first blood sample.

Anticholinergic Assay

Assayists had no knowledge of neuroleptic medication or MMSE scores. This assay technique measures the total antimuscarinic receptor binding potential in human serum based on competition with tritiated quinuclidinyl benzilate ([^{3}H]-QNB) in rat striatal and forebrain receptors. The reliability and validity of this method have been previously discussed.[5,9,14] The assay is calibrated by using atropine at concentrations ranging from 0.10 to 10 nM. This standard curve is linear (r = .99) and its interassay percentage coefficient of variation in our laboratory is 5.6% (based on 5 assays, at 2.5 nM of atropine). The anticholinergic potency of a drug can be indexed by reduction in the known receptor occupancy rates of [^{3}H]-QNB. The radioreceptor assay values are given in atropine equivalent units of picomoles per milliliter (pmol/mL).

RESULTS

Table 1 displays the mean anticholinergic levels (atropine equivalent values) of the clozapine and risperidone groups. Independent t tests indicated that the groups differed in anticholinergic levels at both T1 ($t = 6.3$, $df = 13.7$, $p < .001$) and T2 ($t = 5.8$, $df = 18.1$, $p < .001$). Clearly, these data revealed a multifold difference in the anticholinergic levels induced by these 2 medications.

Dependent t tests revealed that both clozapine and risperidone anticholinergic levels were statistically identical across T1 and T2. Thus, strong stability in the anticholinergic properties of both drugs was observed, at least over the short time period of this study. Lastly, the clozapine and risperidone groups did not differ significantly on MMSE scores, with their group means varying by less than 1 point. Pearson correlation data revealed that MMSE scores were not related to anticholinergic levels at T1 or T2, either in the sample as a whole or when exam-

Table 1. Means and Standard Deviations of Clozapine and Risperidone Groups for Time 1 and 2 Anticholinergic Levels and Mini-Mental State Examination Scores

	Anticholinergic Levels in pmol/mL Atropine Equivalent						Mini-Mental State Examination		
	Time 1			Time 2					
Medication Group	N	Mean ± SD	(range)	N	Mean ± SD	(range)	N	Mean ± SD	(range)
Clozapine	14	4.35 ± 2.38	(1.7–9.3)[a]	15	4.07 ± 2.22	(1.7–9.7)	15	27.40 ± 2.99	(19–30)
Risperidone	7	0.27 ± 0.28	(0.0–0.81)	7	0.43 ± 0.64	(0.0–1.9)	7	26.70 ± 6.13	(13–30)

[a]N = 14 because 1 patient was not available for blood collection at T1.

ined within the 2 medication groups. Finally, there was no indication that psychiatric symptom status was predictive of global cognitive state, as MMSE scores were not correlated with BPRS total scores. Also, the medication groups did not differ in BPRS total scores, suggesting that they were comparable in terms of clinical status.

DISCUSSION

Prior in vitro pharmacologic studies suggest that the anticholinergic properties of clozapine and risperidone differ by large multiples for several human muscarinic receptors (for geometric mean K_d, nM, data on M_1 through M_5).[2] We believe this report to contain the first in vivo data on the anticholinergic effects of these drugs. This study involves a sample of well-defined schizophrenic patients taking a single neuroleptic regimen (i.e., no other rival anticholinergic agents were present); therefore, it provides strong evidence that clozapine stimulates higher human brain anticholinergic activity than risperidone. This is consistent with the data showing that clozapine antagonizes selected muscarinic receptors (M_1, M_2, M_3, and M_5) despite being an agonist at others (M_4).[15] Moreover, the anticholinergic levels induced by clozapine appear to be lower than those observed for standard neuroleptics such as haloperidol even when an adjunctive antiparkinsonian medication is present.[5]

Unlike previously reported relationships between serum anticholinergic levels and general cognitive status, the high anticholinergic activity observed in this study was not associated with cognitive impairment as assessed by the MMSE. For instance, a study by Tune and colleagues[16] measured serum anticholinergic levels and mental status before and after surgery in a sample of open-heart surgery patients with the same assessments used here. They found that levels of 1.5 pmol/sample atropine equivalents were associated with severe cognitive impairment (i.e., delirium), and that 7 of 8 patients delirious after surgery had significantly elevated serum anticholinergic drug levels (> 1.5 pmol/mL). Such data led to the expectation that the anticholinergic levels obtained in this study from patients treated with clozapine would produce significant cognitive impairment. Clearly, however, this was not the case. In fact, only 2 (13.3%) of 15 clozapine patients had an MMSE score in the impaired range (below 24; anticholinergic levels = T1 = 2.94, T2 = 2.99 and T1 = 4.96, T2 = 2.43). By comparison, 1 (16.6%) of 6 risperidone patients had an MMSE score below the cutoff for impairment.

In summary, this study (1) provides in vivo data validating the previous in vitro findings that have drawn a strong distinction between clozapine and risperidone in terms of anticholinergic effects; (2) demonstrates that the anticholinergic levels induced by clozapine (4.1–4.3 pmol/mL) may still be less than those of neuroleptics such as haloperidol when an antiparkinsonian drug is present (approximately 10 pmol/mL) see Tune and Coyle[14]; and (3) indicates that the moderately high anticholinergic levels associated with clozapine are not a sufficient condition for cognitive impairment as detected by the MMSE.

The reason for the lack of association between anticholinergic levels and cognitive impairment is ambiguous. Six possibilities merit consideration: (1) the anticholinergic levels obtained were not high enough to cause cognitive impairment; (2) the MMSE was too gross and insensitive a cognitive measure to detect anticholinergic effects; (3) the schizophrenic illness brought with it a mechanism that reduced the anticholinergic effects of clozapine; (4) deleterious anticholinergic cognitive effects occur only at higher ages; (5) clozapine has other neurochemical effects that nullify its anticholinergic cognitive effects; and (6) the anticholinergic assay was more sensitive to muscarinic antagonist than agonist effects. Studies with medical and/or elderly samples [7–9,16–19] argue against the first 2 possibilities, as the anticholinergic levels obtained here were comparable to or even higher than those in the other studies, and many of these other studies have observed the relationship between high anticholinergic levels and cognitive impairment using the MMSE. The MMSE, however, is poor at detecting both subtle and specific cognitive impairment and will produce false negatives if either is present. For instance, the Goldberg et al.[6] study suggested that clozapine may specifically affect memory. Since the MMSE does not measure memory adequately, our data leave open the possibility of more specific cognitive effects. Our data do point out the absence of severe, more global cognitive effects—phenomena which, as noted previously, have been observed at the anticholinergic levels we found with clozapine. Also, the possible effect of premorbid individual differences on MMSE scores needs

to be acknowledged, although we have no reason to suspect that this factor biased group-level scores on the MMSE. Finally, although the range and variability of MMSE scores appeared reasonable (Table 1), ceiling effects may have occurred. (Note, however, that the scores of the clozapine group were more clustered in the upper range.) The authors are not aware of any evidence supporting the third possibility.

Regarding the fourth alternative, the "anticholinergic model" of severe cognitive impairment or delirium has been best demonstrated in aged samples (for a review see Trzepacz[18]). For instance, the study by Tune and colleagues[16] reporting an association between high anticholinergic levels and delirium involved a sample with a mean age of 55 years (N = 29; range, 29–75 years). Many of the other studies reporting an association also used samples older than the one used here.[7–9,17,19]

Regarding the fifth alternative, the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor agonist and serotonergic (5-HT) antagonistic effects of clozapine have been described.[20,21] The cognitive effects of NMDA activity would seem to stem from enhancement of long-term potentiation, considered a possible neural substrate for learning. The cognitive effects of 5-HT blockade activity are less clear, as most serotonin studies showing affects on cognition have involved reuptake inhibitors.[21] For instance, Flood and Cherkin[22] demonstrated in animals that 5-HT reuptake blockers can both reverse the effects of anticholinergic blockade and enhance memory. The drug ondansetron, a selective 5-HT_3 antagonist, however, has been shown to stimulate cortical release of acetylcholine and improve cognitive performance in animals following scopolamine-induced impairments.[23] Steckler and Sahgal,[24] summarizing how serotonergic and acetylcholine systems interact, highlighted the possibility that 5-HT modulation involves reduction of certain deleterious anticholinergic effects.

Sixth, the tissue used for the serum assay came from rat forebrain and striatal tissue where both M_1 and M_4 receptor subtypes predominate. Nonetheless, radioreceptor assays are more sensitive to antagonists than agonists. Therefore, clozapine's possible affinity for M_4 receptors may not have been apparent. It has been speculated that the selective muscarinic agonism of clozapine at M_4 may account for its sialogogue effects.[15] The role such agonist activity may play in balancing or preventing cognitive impairment, however, has not been explored.

A limitation of this study is the lack of pretreatment anticholinergic and cognitive measures. While all subjects were taking their respective drug regimens for at least 30 days, no data confirm that the medication groups started with equivalent anticholinergic levels. This study does not control for possible differences in anticholinergic levels related to acute versus chronic administration of the anticholinergic medication. Also, the study is hampered by the small sample size and its concomitant power limitations. Finally, it would have been beneficial to have had additional clinical measures of anticholinergic activity, such as salivary flow, as a way of determining the clinical relevance of the serum laboratory data.

CONCLUSION

The data from this study clearly demonstrate that anticholinergicity stands as an important point of difference between clozapine and risperidone with important implications in terms of clinical, particularly cognitive, side effects. These data also make clear that actually observing such deleterious anticholinergic effects is quite complex, and their presence ultimately may be a function of many factors including age, the underlying clinical disorder (schizophrenia), and the other neurochemical effects of the anticholinergic agent. Clearly, despite clozapine's reputation for relatively high anticholinergic activity (e.g., adjunctive antiparkinsonian medication is not needed with clozapine), the actual anticholinergic impact of this drug may be relatively low. Verification of the full clinical and cognitive impact of anticholinergic activity from clozapine and the other atypical neuroleptics awaits further study with a full range of more specific cognitive tests.

Drug names: chlorpromazine (Thorazine and others), clozapine (Clozaril), haloperidol (Haldol and others), risperidone (Risperdal).

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