Effects of clozapine and typical antipsychotic drugs on plasma 5-HT turnover and impulsivity in patients with schizophrenia: a cross-sectional study

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Objective: To compare the efficacy of clozapine with typical antipsychotic drugs in controlling impulsivity and to explore the possible correlation of impulsivity with plasma 5-hydroxytryptamine (5-HT) levels, plasma 5-hydroxyindoleacetic acid (5-HIAA) levels and plasma 5-HT turnover. **Design**: Prospective, cross-sectional study open to medication and blinded to biochemical analyses. **Participants**: Healthy control subjects (n = 24) and 46 inpatients and outpatients meeting the DSM-IV criteria for schizophrenia; 20 were being treated with clozapine and 26 were taking typical antipsychotic drugs. **Interventions**: All psychotropic drugs other than clozapine or typical antipsychotic drugs were discontinued for at least 5 days and subjects fasted overnight before they were assessed. **Outcome measures**: Coccaro Impulsivity Scale scores, plasma 5-HT levels, 5-HIAA levels and 5-HT turnover. **Results**: Patients treated with clozapine and those treated with typical antipsychotics had significantly higher impulsivity scores than the control group, and the mean impulsivity score of the typical antipsychotic group was significantly higher than that of patients treated with clozapine. The mean concentration of 5-HT of the typical antipsychotic group was significantly lower than that of the control group and patients treated with clozapine; however, mean plasma levels of 5-HIAA were significantly higher for the clozapine group than the other 2 groups. 5-HT turnover was significantly higher for the 2 drug-treatment groups than for the control group. **Conclusions**: These results suggest that treatment with clozapine should be considered for patients with schizophrenia who are impulsive and aggressive.

Objectif : Comparer l'efficacité de la clozapine à celle de neuroleptiques typiques lorsqu'il s'agit de contrôler l'impulsivité et explorer le lien possible entre l'impulsivité et les taux plasmatiques de 5-hydroxytryptamine (5-HT), les taux plasmatiques d'acide 5-hydroxyindoleacétique (5-HIAA) et le renouvellement de

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Medical subject headings: antipsychotic agents; clozapine; impulsive behavior; schizophrenia; serotonin

J Psychiatry Neurosci 2000;25(4):347-52.

Submitted Sept. 17, 1999
Revised Dec. 12, 1999
Accepted Feb. 24, 2000

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la 5-HT dans le plasma. **Conception** : Étude transversale prospective ouverte aux médications et à l'insu d'analyses biochimiques. **Participants** : Sujets témoins en bonne santé (n = 24) et 46 patients en service interne et externe satisfaisant aux critères DSM-IV pour la schizophrénie : 20 étaient traités à la clozapine et 26 prenaient des neuroleptiques typiques. **Interventions** : On a cessé d'administrer tous les psychotropes autres que la clozapine ou des neuroleptiques typiques pendant au moins cinq jours et les sujets étaient à jeun depuis 12 heures avant l'évaluation. **Mesures de résultats** : Résultats selon l'échelle de l'impulsivité de Coccaro, taux plasmatiques de 5-HT, taux de 5-HIAA et renouvellement de la 5-HT. **Résultats** : Les patients traités à la clozapine et ceux qui étaient traités aux neuroleptiques typiques ont obtenu des résultats beaucoup plus élevés selon l'échelle de l'impulsivité que les sujets témoins et les résultats moyens selon l'échelle de l'impulsivité du groupe de sujets qui prenaient des neuroleptiques typiques ont été beaucoup plus élevés que ceux des patients traités à la clozapine. La concentration moyenne de 5-HT chez les sujets traités aux neuroleptiques typiques était beaucoup plus basse que celle des sujets témoins et celle des patients traités à la clozapine. Les taux plasmatiques moyens de 5-HIAA étaient toutefois beaucoup plus élevés chez les sujets traités à la clozapine que chez ceux des deux autres groupes. Le renouvellement de la 5-HT était beaucoup plus élevé chez les sujets des deux groupes traités aux médicaments que chez ceux du groupe témoin. **Conclusions** : Ces résultats indiquent qu'il faudrait envisager le traitement à la clozapine chez les patients atteints de schizophrénie qui manifestent de l'impulsivité et de l'agressivité.

Introduction

The management of impulsive, aggressive and violent patients with schizophrenia is extremely difficult and often unsatisfactory. Management approaches that include seclusion and physical restraint are emotionally and financially costly. Emotionally, these events and those leading up to them disturb not only the patient, but also other patients on the ward and the staff who must impose seclusion or restraint. Financially, they are a possible source of injury for staff and other patients. Pharmacological restraint using typical antipsychotic drugs also appears to be unsatisfactory. However, evidence suggests that treatment with clozapine, an atypical antipsychotic drug, reduces the number and duration of restraint and seclusion episodes, as well as suicidal and aggressive behaviour, in patients with schizophrenia.[1]

The unique mechanism of action of clozapine is not clear, although a serotonergic mechanism has been suggested.[2-6] Indeed, serotonin (5-hydroxytryptamine [5-HT]) has been implicated in a variety of behaviours and somatic functions that are disturbed in patients with schizophrenia (i.e., perception, attention, mood, aggression, impulsiveness, hostility, sexual drive, appetite, motor behaviour and sleep). Given the complexity of the 5-HT system and its interactions with other neurotransmitters, it seems likely that disturbances of the 5-HT system may play a role in specific symptoms of schizophrenia. In a review of the role of 5-HT in schizophrenia, Bleich and colleagues[7] concluded that alterations in brain 5-HT metabolism could be related to Crow's concept of type II (negative syndrome) schizophrenia and that the 5-HT abnormality "might involve 5-HT postsynaptic receptor hypersensitivity." They reviewed the data before 1988 on levels of platelet and whole blood 5-HT and concluded that platelet or whole blood concentrations did not differ between patients with schizophrenia and controls. In contrast, significant positive correlations between platelet and blood 5-HT levels and cortical atrophy, as well as auditory hallucinations, have been reported. Bleich and others[7] and Braunig and others[8] reported decreased blood concentrations of 5-HT in female patients with schizophrenia who were suicidal. Increased platelet 5-HT concentrations were found in patients with schizophrenia with paranoid features, but a decrease was found in patients with non-paranoid features.[9]

However, the relationship between peripheral 5-HT metabolism, impulsivity and antipsychotic drug treatments for patients with schizophrenia is unclear. We therefore investigated the correlation between impulsivity, 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT turnover and typical and atypical antipsychotic (i.e., clozapine) drug treatment in patients diagnosed with schizophrenia. These results were compared with those from healthy controls.

Methods

Subjects

Three groups were recruited for the study. The control subjects (n = 24, male:female ratio [m:f] = 15:9) were recruited mainly from the hospital staff. All were in

good physical health, and medical and neuropsychiatric evaluations showed that all measures were within normal limits. Subjects were excluded if there was evidence of current alcohol or drug use or a history of alcohol or drug abuse. They also had to be psychotropic drug free for at least 3 months and free of ASA, paracetamol and contraceptives for at least 5 days before they were assessed.

Patients in the 2 drug-treatment groups were recruited from the inpatient and outpatient rehabilitation services at Leicester hospitals. In total, 46 patients with schizophrenia were entered into the study according to a simple stratification scheme to give approximately equal numbers of patients treated with clozapine, patients treated with typical antipsychotic drugs and control subjects, balanced for age and sex. None of the patients had taken any additional psychotropic drugs for at least 5 days before they were assessed.

Patients treated with clozapine (n = 20, m:f = 10:10; refractory patients with schizophrenia) had failed in the past to respond to at least 3 typical antipsychotic drug treatments of at least 8 weeks (mean dosage of clozapine and standard error of the mean [SEM] = 360 [SEM 50] mg/day).

Patients treated with typical antipsychotic drugs (n = 26, m:f = 15:11; treatment-responsive patients) had fully or partially responded to typical antipsychotic drug treatment and were not receiving clozapine. The mean chlorpromazine dosage equivalent of the antipsychotic medication was 600 (SEM 64) mg/day.

All subjects gave consent to participate in this study. All subjects fasted overnight before they were tested.

Patient selection criteria

Patients were included in the study if:

- their clinical diagnosis of schizophrenia was consistent with criteria in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV), and they had been ill for at least 2 years,
- were receiving routine treatment for schizophrenia,
- they had already received at least 20 weeks of therapeutic antipsychotic treatment,
- they could continue with routine anticholinergic treatment

Patients were excluded from the study if:

- there was drug use or chronic use of any other medication that could affect plasma 5-HT concentrations (e.g., anxiolytics, antidepressants, mood stabilizers, addictive drugs) in the 3 months before the study
- there was evidence of chronic alcoholism (i.e., dependence, abuse or withdrawal symptoms)
- there was clinical evidence of central nervous system infection or malignancy
- they had any medical disorder that could affect plasma 5-HT concentrations (e.g., pheochromocytoma, platelet dysfunction)

Assessment of impulsiveness

The Coccaro Impulsiveness Scale[10,11] was used to rate the level of impulsiveness; clinical scores ranged from 0–10 as follows: 0 = no impulsiveness; 1–3, verbal impulsiveness; 4–5, physical impulsiveness and aggression against objects; 6–7, physical impulsiveness and aggression against self; 8–9, physical impulsiveness and aggression against others; 10, criminal impulsiveness and violent acts.

Biochemical determination of plasma 5-HT and 5-HIAA levels

A 9-mL blood sample was taken from each subject after an overnight fast, and 5-HT and 5-HIAA concentrations were determined immediately in "duplicate" (see the methodology in Whitaker et al.[12] and Dursun et al.[13]). Each blood sample was immediately mixed with 1 mL of 3.13% trisodium citrate solution and centrifuged for 10 minutes at 1200 g; 1 mL of the supernatant was either analyzed immediately or frozen at –70ºC until it was analyzed, usually within 72 hours.

Plasma 5-HT and 5-HIAA levels were determined by an HPLC-fluorescent detection method,[14] and 5-HT turnover was calculated as [5-HIAA]÷[5-HT]. Those performing the analyses were blind to subject status. Samples were thawed at room temperature and mixed, and 0.2-mL aliquots of 10% trichloroacetic acid (TCA) were slowly added to 0.2 mL of the sample. The mixture was then vortexed for 10 seconds and placed on iced water for 30 minutes. A final centrifugation step of 10 minutes at 1200 g was performed, and supernatant was injected directly on the HPLC system. All samples were assayed in duplicate.

Standards (5-HT and 5-HIAA) and all other reagents and chemicals were purchased from Sigma (Dorset, UK) unless otherwise specified. Stock standards of 1 mg/mL of both standards were prepared in 10% TCA and stored at –70°C. Working standards were prepared

by diluting stock standards of 5-HT and 5-HIAA in 5-HT/5-HIAA-free plasma samples on the day of analysis. 5-HT and 5-HIAA levels in the samples were compared with standards and calculated by peak height.

An isocratic HPLC method was developed for the separation and quantitation of 5-HT and 5-HIAA. Separation occurred on a Spherisob ODS column (150 mm × 4.6 mm); the mobile phase consisting of 0.1 M ammonium acetate and 0.1 g/L octane sulphonic acid was adjusted to a pH of 5.1 with acetic acid before methanol was added (final concentration 10% v/v). The flow rate was 1.2 mL/min. The detection system was fluorescence spectroscopy using a Perkin Elmer 3000 fluorimeter.

Statistical analyses

Data are expressed as means and SEMs. Differences between the control group, the typical antipsychotic drug group and the clozapine group were assessed with a 1-way analysis of variance and post hoc Student Neuman-Keuls tests using SPSS. Exploratory analyses were performed to determine if there were any significant correlations between impulsivity scores and 5-HT or 5-HIAA concentrations or plasma 5-HT turnover in each of the drug-treated groups; non-parametric Spearman's rank-order correlation coefficients (2-tailed) were used.

The study was approved by the ethics committees of the hospitals involved and was performed in accordance with the ethical standards set forth in 1964 Declaration of Helsinki.

Results

Mean clinical impulsivity scores (and SEMs) were: 0 (SEM 0) for healthy controls, 3.8 (SEM 0.7) for patients treated with clozapine and 7.1 (SEM 0.4) for those treated with typical antipsychotic drugs. Both of the groups of patients with schizophrenia had significantly higher impulsivity scores than the control group, and the mean impulsivity score of the typical antipsychotic group was significantly higher than that of the patients treated with clozapine (Table 1).

Plasma 5-HT and 5-HIAA concentrations and 5-HT turnover for each group are presented in Table 1. The mean concentration of 5-HT of the typical antipsychotic group was significantly lower than that of the control group and patients treated with clozapine; however, mean plasma levels of 5-HIAA were significantly higher for the clozapine group than the other 2 groups. 5-HT turnover was significantly higher for the 2 groups of patients with drugs than for the control group.

There was no correlation between the impulsivity score and 5-HT turnover ($r = 0.281$, $p = 0.275$), 5-HT concentration ($r = 0.063$, $p = 0.811$) or 5-HIAA concentration ($r = 0.088$, $p = 0.736$) in the group treated with clozapine. In the typical antipsychotic group, there was no significant correlation between impulsivity score and 5-HT concentration ($r = 0.256$, $p = 0.238$) or 5-HIAA concentration ($r = 0.059$, $p = 0.789$), but there was a trend toward a correlation between impulsivity score and 5-HT turnover; it did not reach a statistical significance, however ($r = 0.488$, $p = 0.057$).

Discussion

These results further demonstrate that patients with schizophrenia are more impulsive than healthy controls and that patients treated with clozapine score significantly lower on impulsivity scales than those treated with typical antipsychotic drugs. Indeed, the unique anti-impulsive action of clozapine may be due to its effects on the 5-HT system — the clozapine group had significantly higher plasma levels of 5-HT and 5-HIAA

Table 1: Effects of clozapine and typical antipsychotics on impulsivity scores and plasma 5-HT turnover in patients with schizophrenia

	Mean (and standard error of the mean)					
Treatment group	Age, yr	Dose, mg/day	Impulsivity score	Plasma 5-HT level, ng/mL	Plasma 5-HIAA level, ng/mL	Plasma 5-HT turnover
Control	36 (2.2)	Drug free	0 (0)	5.8 (0.3)	9.5 (0.5)	1.7 (0.1)
Clozapine	36 (1.9)	360 (50)	3.8 (0.7)*†	5.8 (0.6)†	12.5 (1.1)*†	2.9 (0.4)*
Typ-APD	40 (1.7)	600 (64)	7.1 (0.4)*	3.9 (0.6)*	8.9 (0.7)	2.3 (0.3)*

Typ-APD = typical antipsychotic drugs.
*Significantly different from the mean of the control group; $p < 0.05$, 1-way analysis of variance (ANOVA) and post-hoc Student Neuman-Keuls test.
†Significantly different from mean of typical antipsychotic group; $p < 0.05$, 1-way ANOVA and post-hoc Student Neuman-Keuls test.

than the typical antipsychotic drug group and also showed a significant increase in 5-HT turnover. It is interesting to note, however, that although the plasma levels of 5-HT for the group treated with clozapine were similar to those of the control group, patients treated with clozapine had significantly higher impulse scores than the control group. This suggests that other neurotransmitters and factors are also involved.

Our data on impulsivity scores support previous reports of a reduction in the number of restraints and seclusions,[1] of suicidal behaviour[15] and of aggressive behaviour[16] after patients with treatment-resistant schizophrenia are treated with clozapine. The importance of clozapine treatment for such patients with schizophrenia lies in its possible benefits, the most important of which is likely an improvement in quality of life for themselves, staff, other patients, families, relatives and all others involved in the care of these patients.

Plasma 5-HT concentrations were significantly higher in patients treated with clozapine. These data are consistent with other studies in which increased blood 5-HT concentrations[17] and cerebral spinal fluid and urine 5-HIAA concentrations[18] were reported after clozapine treatment. However, the unique mechanisms responsible for the anti-impulsive effects of clozapine are not clear.

There is consistent evidence that impulsive, aggressive and suicidal behaviours are linked to quantitative dysregulation in central 5-HT systems. Regardless of the underlying mechanisms, low serotonergic functioning seems to be associated with an increased vulnerability to a range of psychopathology.[7-9] The correlation between low plasma and platelet 5-HT concentrations and impulsive or aggressive behaviour[7-9] is further supported by studies on personality disorders,[19] major depressive disorder[20,21] and aggressive feelings and acts, which have been shown to be significantly related to low cerebral spinal fluid 5-HIAA concentrations.

Therefore, one possibility, supported by our results, is that clozapine may increase plasma 5-HT and 5-HIAA concentrations and, via a similar effect in the brain, reduce impulsiveness in patients with schizophrenia. However, the mechanism by which clozapine increases plasma 5-HT and 5-HIAA concentrations remains to be determined. It may be that clozapine increases plasma-free tryptophan but reduces total tryptophan concentration. Increased plasma-free tryptophan would be expected to enhance brain 5-HT synthesis, since the availability of tryptophan in the brain is believed to be the rate-limiting step in 5-HT synthesis.[22]

Norepinephrine may also be important in the mechanism of action of clozapine on impulsiveness in schizophrenia.[23] Our study is therefore limited by the fact that we did not also determine norepinephrine and 3-methoxy-4-hydroxyphenylglycol levels and norepinephrine turnover in our samples. In addition, patients taking clozapine represent a rather "severe" group in terms of psychopathology. The impact of severe psychopathology and unresponsiveness to typical antipsychotic drugs on 5-HT systems remains unclear. Furthermore, since this is a cross-sectional study, initial impulsivity scores were not obtained. Prospective randomized studies are required to avoid these limitations.

Our results suggest that treatment with clozapine should be considered for patients with schizophrenia who are impulsive and aggressive. However, further research is required to understand the mechanism of action of clozapine, especially on the central the 5-HT system, in relation to impulsivity in patients with schizophrenia.

Acknowledgements

We thank Ms. Diane Bird and Ms. Helen Tremayne for their review of this article and for their helpful comments. We are grateful to the Leicestershire Mental Health Services NHS Trust for financial support.

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