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Blood biogenic amines during clozapine treatment of early-onset schizophrenia

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Summary. The aims of this investigation were to evaluate long-term and short-term effects of clozapine-treatment on plasma biogenic amines and psychopathology measures in adolescents with schizophrenia (DSM-III-R criteria). The long-term study was conducted in a study sample of 40 young patients (age 14–22 years) following a mean of 3.4 years of neuroleptic treatment. During the study, 20 patients received clozapine, and the other 20 patients were treated with standard neuroleptic medications. At the beginning of the open clinical trials, the patients had already been receiving clozapine treatment for 24 ± 15 months. Assessment of the biochemical and psychopathological measures was performed on six occasions at consecutive 6-week intervals during maintenance treatment with clozapine or conventional neuroleptics. Blood levels of serotonin, 3-methoxy-4-hydroxy-phenylglycol (MHPG), norepinephrine, and epinephrine were significantly higher in clozapine-treated patients than in conventionally treated patients. During long-term treatment, higher serotonin levels were associated with significantly fewer negative symptoms of schizophrenia, whereas higher MHPG levels were correlated with less depression. The short-term effects of clozapine were assessed in a second and independent study sample. After failing on conventional neuroleptics in clinical trials lasting a mean of 1.6 years, 15 inpatients (aged 11-20 years) received clozapine. Weekly ratings of psychopathological symptoms using standard rating scales were performed in parallel to blood samplings for measurements of biogenic amines and serum levels of clozapine. These measures were obtained for 6 weeks during conventional neuroleptic treatment and for 6 weeks during the open-label clozapine trial. Serum levels of serotonin and plasma norepinephrine levels were significantly higher during treatment with clozapine than during pretreatment with typical neuroleptics. A comparison of plasma epinephrine levels in responders (n = 7) and nonresponders (n = 8) to clozapine revealed that response to clozapine can be predicted by epinephrine levels prior to initiation of treatment with clozapine (responders ranging from 32.2 to 90.3 pg/ml; nonresponders ranging from 92.5 to 473.5 pg/ml). Additionally, subjects who responded to clozapine

showed increased mean plasma concentrations of MHPG and epinephrine during treatment with this drug in comparison to the levels measured during pretreatment with typical neuroleptic medication. Nonresponders to clozapine failed to show this increase. Finally, in responders to clozapine a negative linear relationship between negative symptoms of schizophrenia and the concentrations of plasma norepinephrine and serum serotonin were observed. In conclusion, our results demonstrate that plasma epinephrine levels prior to initiation of clozapine therapy predict response to this atypical neuroleptic. Our findings derived from short-term and maintenance treatment with clozapine suggest involvement of norepinephrine, epinephrine and serotonin in the therapeutic actions of the atypical neuroleptic clozapine.

Keywords: Serotonin, norepinephrine, epinephrine, early-onset, schizophrenia, clozapine, human

Introduction

About 30–40% of children and adolescents with schizophrenia are refractory to conventional neuroleptic treatment (Remschmidt et al., 1991). For these patients, a trial of the atypical neuroleptic clozapine can be considered, if strict guidelines for clinical management of clozapine are followed (Remschmidt et al., 1994a,b). Several uncontrolled studies have provided evidence that clozapine might be effective in children and adolescents with treatment-refractory schizophrenia (Birmaher et al., 1992; Blanz and Schmidt, 1993; Frazier et al., 1994; Gordon et al., 1994; Mozes et al., 1994; Piscitelli et al., 1994; Remschmidt et al., 1994; Siefen and Remschmidt, 1986; Towbin et al., 1994). Nonetheless, about 30% of adolescents with treatment-resistant schizophrenia do not respond to clozapine in open-label treatments (Remschmidt et al., 1992). Therefore, the treatment of patients with drug-resistant schizophrenia is a major challenge for the clinician and requires further systematic research.

Measurement of biogenic amines and their metabolites in cerebrospinal fluid (CSF) and plasma is considered a promising tool to help elucidate the mechanisms of action of neuroleptics (Kahn and Davidson, 1993). Only a few studies compared the neurochemical effects of clozapine with those of conventional neuroleptic drugs (Ackenheil, 1989; Banki, 1978; Breier et al., 1994; Green et al., 1993; Pickar et al., 1992).

The major disturbances in the brain of schizophrenics regard the dopaminergic neurotransmission, especially of the mesolimbic-cortical system (van Kammen, 1991; van Kammen and Kelley, 1991). Davis et al. (1991) summarize the findings on the role of the dopaminergic system in schizophrenia, showing high dopamine and -metabolite concentrations in various subcortical brain regions together with an increase in dopamine receptor binding, combined with a lower prefrontal dopamine activity. Plasma levels of dopamine metabolites seem to be a good correlate for the disease and the activtiy of neuroleptics. Plasma MHPG levels correlate with disturbances of the central noradrenergic system, which modulates the dopaminergic function (van Kammen and Kelley, 1991). A major role in the pathology of the noradrenergic

system could play the locus coeruleus, the major source of noradrenergic neurons in the human brain (Orellana-Vidal, 1995). The catecholaminergic dysfunction in schizophrenia led in the study of Meszaros et al. (1996) to investigate the dopamine-beta-hydroxylase gene as possible candidate gene for schizophrenia. Though this is not the case, the catecholaminergic dysfunction includes besides the dopaminergic system also noradrenergic neurotransmission (Yamamoto et al., 1994).

The role of 5-HT in schizophrenia (Kapur and Remington, 1996) is supported by several different findings, coming from postmortem studies, measures of CSF and peripheral 5-HT, and binding studies with antipsychotic drugs (Breier, 1995). 5-HT receptor antagonists, especially for 5-HT_2 receptor subtype have been reported to ameliorate negative symptoms (Brunello et al., 1995). Clozapine a potent anti-schizophrenic drug, shows besides its dopamine receptor antagonism a high efficacy as a 5-HT_2 receptor antagonist (Brunello et al., 1995). Another antipsychotic drug, Ziprasidone, has been shown to have high affinity to various 5-HT receptors such as the 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, and 5-HT_{2C} subtypes respectively (Seeger et al., 1995). Post mortem studies give evidence for a reduction of 5-HT_{2A} receptor subtype in the prefrontal cortex, combined with an increase of the 5-HT_{1A} receptor (Arora and Meltzer, 1991).

The aim of the present study was to measure blood levels of serotonin and of catecholamines in adolescents with schizophrenia during maintenance therapy (study-I) and during short-term treatment (study-II) with the atypical neuroleptic clozapine.

Material and methods

Subjects

Maintenance treatment (study I)

For both open clinical trials written informed consent was obtained from the patients and their parents. Patients with secondary diagnosis of substance abuse were excluded from the study. To evaluate the long-term effects of clozapine, a study of 40 adolescent patients (age 14–22 years) with schizophrenia was conducted following a mean of 3.4 years of neuroleptic treatment (study-I). This prospective trial included all 40 patients with DSM-III-

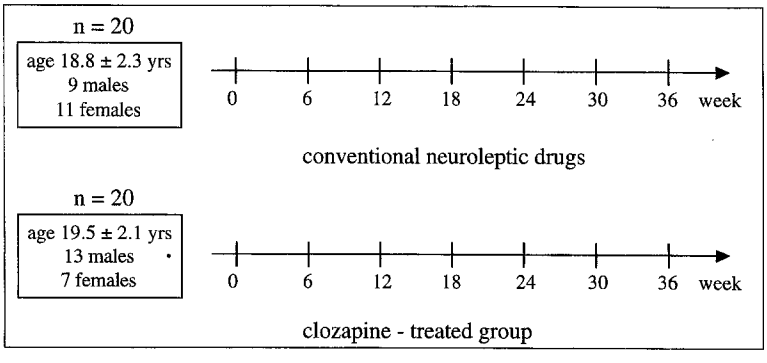

Fig. 1. Study group with long-term treatment of clozapine vs. conventional neuroleptic drugs

R criteria of schizophrenic or schizoaffective psychosis in a rehabilitation center ("Leppermühle") for children and adolescents with schizophrenia. The data collection was performed on six occasions at consecutive 6-week intervals during maintenance treatment (Fig. 1). During the study, 20 patients with schizophrenia received clozapine, and the other 20 patients were treated with standard neuroleptic medications. At the beginning of the prospective investigation, the age range of the total sample was 14–22 years. There were no statistically significant differences (Chi-square or Fishers test) between the two treatment groups with respect to gender or age distribution (total sample 19.1 ± 2.2 years, clozapine group 19.5 ±2.1 years, conventionally treated group 18.8 ± 2.3 years). According to DSM-III-R criteria, the following subtypes of schizophrenic psychoses were found in the total sample of 40 subjects: paranoid type (17 males, 77%; 10 females, 55%), disorganized type (5 males, 22%; 4 females, 22%), residual type (1 female, 5%), and schizoaffective disorder (3 females, 16%). At the start of the investigation, the course of the illness showed the following distributions, using DSM-III-R criteria: subchronic 12.5% (n = 5), chronic 72.5% (n = 29), chronic with acute exacerbation 7.5% (n = 3), and remitted 7.5% (n = 3). Of the sample of 40 young patients, 32 patients (80%) were already in a chronic state (with or without acute exacerbation). The diagnoses, age and gender distribution (22 males, 18 females) revealed no significant differences, as determined by chi-square or Fishers test. Intellectual functioning by standard intelligence tests (Wechsler Intelligence Scale or Culture Fair Test) showed a high IQ level (IQ 115–129) in 5% of patients (n = 2), normal range (IQ 85–114) in 58% (n = 23), low normal (IQ 70–84) in 35% (n = 14), and mild mental retardation (IQ 50–69) in 2% (n = 1). Indications for clozapine treatment were nonresponse, aggravation of target symptoms, or severe side effects during treatment with typical neuroleptic drugs. These adolescents were first treated for schizophrenia with conventional neuroleptic medication at age 15.7 ± 1.5 years, and clozapine was initially administered as neuroleptic monotherapy at age 17.5 ± 2.0 years. At the beginning of the prospective trial, the patients had already been receiving clozapine treatment for 24 ± 15 months.

The 20 adolescents, receiving conventional neuroleptic therapy were treated with haloperidol (n = 9, 22.5%), levomepromazine (n = 5, 12.5%), fluphenazine (n = 4, 10%), flupentixole (n = 3, 7.5%), chlorprothixene (n = 2, 5%), promethazine (n = 1, 2.5%), and thioridazine (n = 1, 2.5%). Of the 20 patients receiving typical neuroleptic agents, 7 received monotherapy and 13 were treated with more than one neuroleptic drug. In the clozapine-treated group, 4 patients (20%) required comedication with sympathomimetics or β-blockers to manage side effects (hypotonia, tachycardia). In the conventionally treated group, 15 patients (75%) experienced side effects that led to comedication with β-blockers, sympathomimetics, biperiden, or laxatives.

Short-term treatment (study II)

To evaluate the short-term effects of clozapine a second open clinical trial was conducted (study-II). The study included all 15 patients out of a total sample of 40 consecutively admitted (October 1991- May 1994) inpatients with schizophrenia (DSM-III-R, APA, 1987) who received clozapine because of nonresponse to pretreatment with conventional neuroleptics. According to DSM-III-R criteria, the following subtypes of schizophrenia were found in the sample of 15 subjects: paranoid type (9 males and 5 females) and residual type (1 male). At the beginning of the prospective investigation, the age range of the sample was 11–20 years, with a mean of 17.3 (SD = 2.2) years (males 17.4 years, SD = 2.6; females 17.1 years, SD = 1.3). Intellectual functioning by standard intelligence tests (Wechsler Intelligence Scale or Culture Fair Test) showed a normal range (IQ 85-114) in 53% of patients (n = 8; 5 males, 3 females), low normal range (IQ 70-84) in 27% (n = 4; 3 males, 1 female), and mild mental retardation (IQ 50-69) in 20% (n = 3; 2 males, 1 female). At intake, the course of the illness showed the following distributions according to DSM-III-R criteria: subchronic 40% (n = 6), chronic 13.3% (n = 2), subchronic with acute exacerbation 6.6% (n = 1), chronic with acute exacerbation 20% (n = 3), in re-

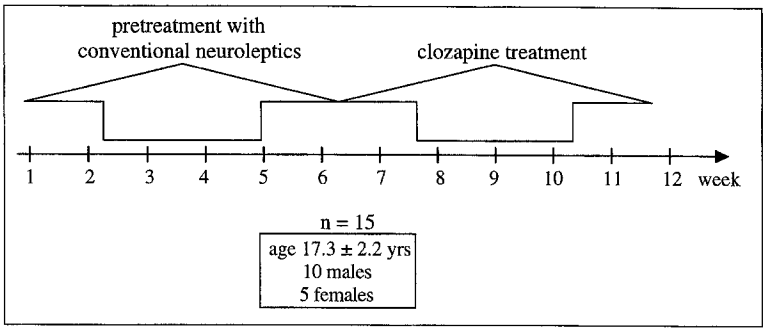

Fig. 2. Study group with short-term treatment of clozapine

mission, but with severe side effects due to conventional neuroleptics 6.6% (n = 1), and unspecified 13.3% (n = 2). The mean age at diagnosis of DSM-III-R schizophrenia for this sample was 15.6 (SD = 1.9) years (males 15.4 years, SD = 2.1; females 16.1 years, SD = 1.4). At that age, the patients experienced characteristic psychotic symptoms, and they were first hospitalised with the diagnosis of schizophrenia at a mean age of 16.1 (SD = 1.6) years (males 15.9 years, SD = 1.9; females 16.3 years, SD = 1.2). In all 15 adolescents conventional neuroleptic medication was initiated at a mean age of 15.7 (SD = 1.8) years (males 15.6 years, SD = 1.9; females 16.0 years, SD = 1.7). At intake, the 15 patients received neuroleptic therapy, as follows: fluphenazine (47%), haloperidol (47%), levomepromazine (33.3%), promethazine (26.7%), chlorprothixen (13.3%), benperidol (13.3%), and perphenazine (6.6%). Three patients received monotherapy with neuroleptics and 12 required comedication with biperiden, sympathomimetics, beta-blockers or laxatives to manage side effects. The patients were followed-up for six weeks of consecutive treatment with the above mentioned neuroleptics (Fig. 2). Thereafter, the study sample received clozapine because of nonresponse (n = 11, 72%) and/or severe side effects (n = 13, 87%). Clozapine was administered in escalating oral doses, starting with 25 mg/daily (1st week: 25–50 mg; 2nd week: 50–200 mg; 3rd-4th week: 100–400 mg; 5th-6th week: 100–600 mg). The individual optimal dose of clozapine was achieved after 5 to 6 weeks of clozapine treatment. Therefore, group comparisons between pretreatment with typical neuroleptics and clozapine treatment were carried out using data from weeks 5–6 (conventional neuroleptics), and weeks 11–12 (5th–6th week of clozapine medication), respectively. We categorised the schizophrenic patients as responders and nonresponders according to two response criteria: (1) a 20% drop in BPRS total score and (2) a final minimum BPRS score of 34 or less. These measures are in agreement with response criteria used in recently performed studies on neuroleptic treatment, response prediction and outcome (e.g. Kronig et al., 1995; Miller et al., 1994; Potkin et al., 1994).

HPLC analysis of biogenic amines and metabolites

Plasma catecholamines, plasma level of 3-methoxy-4-hydroxyphenylglycol (MHPG), and serum serotonin concentrations were measured as previously described (Schulz et al., 1996) by HPLC kits (Catechol-Kit No. 5000, Serotonin-Kit No. 3000) obtained from Chromsystems Corporation (Munich, Germany).

The biochemical probes were obtained using standard procedures for pre-study diet, blood collection, and storage of biogenic amines (Lake and Ziegler, 1985; Pluto and Bürger, 1988; Weier et al., 1986; Ganhao et al., 1991; Boomsma et al., 1993). For the determination of the catecholamines, blood was drawn from an antecubital vein and immediately injected into EGTA Kabevetten (KABE Labortechnik, Nümbrecht/Elsenroth, Germany).

Blood samples for serotonin determination were collected in serum-monovettes (Sarstedt, Germany) by venipucture.

Normal range values of the biogenic amines were derived from data obtained by Chromsystems Corp. (Munich, Germany), which were obtained by the HPLC method used in our study. The following ranges were applied: serum serotonin 128.4–173.7 ng/ml, plasma MHPG 4.4–6.0 ng/ml, plasma norepinephrine 198.6–268.7 pg/ml, plasma epinephrine 76.0–102.9 pg/ml, and plasma dopamine 77–116.4 pg/ml.

HPLC analysis of clozapine and its major metabolites

Measurements of clozapine, clozapine N-oxide, and norclozapine were conducted using the HPLC method developed by our group (Schulz et al., 1995). Blood samples were taken prior to the intake (morning dose) of the neuroleptic medication (Riederer and Laux, 1992). Blood sampling was performed under strict steady-state conditions, i.e., 4 to 5 half-life intervals after the last change of dosage. For the measurements of clozapine, after coagulation at ambient temperature for 15 min, the blood samples were centrifuged for 10 min at 2500 g and 4°C, and serum was stored into micro test tubes at –80°C until analysed.

Instruments

Positive and negative symptoms of schizophrenia were evaluated using the Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS) developed by Andreasen (1982, 1984a,b). We found interrater reliabilities of both the SANS and the SAPS with kappa values of 0.8 comparable with the values reported in the literature (Andreasen et al., 1991; Moscarelli et al., 1987). As described elsewhere (Remschmidt et al., 1991), the attribution of attentional impairment by the SANS to negative symptoms (rather than also to positive symptoms) seems to be problematic, leading us to exclude this item from the rating scale. The summary scores for the ratings of negative and positive symptoms were calculated according to Andreasen (1982).

In addition to the assessment of positive and negative symptoms ratings, the Brief Psychiatric Rating Scale (BPRS, Overall and Gorham, 1962) was employed to measure symptomatology and outcome during the prospective investigation. In our study, the BPRS Total score and the BPRS Depressive score (including items 1, 2, 5, and 9) were employed for statistical analysis. The clinical ratings were performed during the short-term trial (study-II) weekly and during the long-term trial (study-I) at 6-week intervals by an experienced child and adolescent psychiatrist in parallel to the blood samplings for the biochemical and the pharmacological investigations.

Statistics

Several group comparisons were conducted using chi-square, Student t-test and Fishers exact test. Spearman correlation, median test and Wilcoxons signed rank test for the differences of paired observations were used. For statistical analysis of the biogenic amine concentrations at the various time points repeated measures analysis of variance was conducted (repeated measures ANOVA design, SAS 6.09 for Open VMS).

Results

During long-term treatment (study-I), the individual mean values for the biochemical measures, calculated from six measurements at consecutive 6-week intervals, demonstrate a significant difference between the clozapine-treated patients and the group receiving typical neuroleptic drugs (Table 1). The plasma levels of norepinephrine, MHPG, and the serum levels of serotonin were significantly increased in the clozapine-treated group as compared with the conventionally treated patients.

Table 1. Biochemical measures obtained during long-term treatment (study-I): clozapine vs. typical neuroleptics

	Typical neuroleptics n = 20	Clozapine n = 20	Statistics
Serotonin (ng/mL)	146 ± 77.1	237 ± 110	F value = 9.44, df = 1, $p = 0.0042$**
MHPG (ng/mL)	2.09 ± 1.28	3.98 ± 0.94	F value = 19.37, df = 1, $p = 0.0001$***
Norepinephrine (pg/mL)	445 ± 201	754 ± 292	F value = 15.75, df = 1, $p = 0.0004$***
Epinephrine (pg/mL)	83.0 ± 58.2	104 ± 51.4	n.s.
Dopamine (pg/mL)	122 ± 62.0	153 ± 91.1	n.s.

Individual values (mean ± SD) were calculated from six measurements at consecutive 6-week intervals. Statistical analysis of the amine concentrations in the two treatment groups (typical neuroleptics vs clozapine-treated group) was performed by repeated measures analysis of variance (ANOVA). ** = $p \leq 0.01$, *** = $p \leq 0.001$

Interestingly, patients whose serotonin levels were 50% higher than the upper limit of the normal range had significantly (median test $p = 0.041$) lower scores of negative symptoms on the SANS. Also, a significant relationship was found between plasma MHPG and depressive symptomatology (BPRS). Patients with plasma MHPG levels that were 50% above the upper normal range limit showed significantly (median test $p = 0.029$) lower scores of depressive symptomatology.

To evaluate the influence of short-term neuroleptic medication on the plasma concentrations of catecholamines and the serum levels of serotonin, individual mean values were calculated from the two consecutive measurements at weeks 5 and 6 (typical neuroleptic treatment) and were compared with those during clozapine treatment at weeks 11 and 12. Clozapine differs from typical neuroleptics by increasing plasma norepinephrine as well as serum serotonin levels (Table 2). In addition, plasma concentrations of epinephrine were somewhat higher during clozapine treatment ($p = 0.083$).

Individual mean values of two measurements at weeks 5 and 6 during typical neuroleptic treatment and at weeks 5 and 6 of clozapine administration, demonstrate a reduction of positive symptoms according to SAPS ($p = 0.014$), a decline in BPRS-derived depressive symptoms ($p = 0.011$) as well as a decrease in total BPRS scores ($p = 0.012$) during clozapine treatment. For the negative symptoms of schizophrenia (SANS), a trend towards lower scores can be observed during the trial with clozapine ($p = 0.127$).

With respect to the two response criteria (a 20 % drop in total BPRS scores and a final minimum BPRS score of 34 or less), 7 schizophrenic patients were classified as responders and 8 patients as nonresponders to clozapine treatment. As compared with the symptomatology at intake, responders to clozapine showed a decline in total BPRS scores ranging from 39.3 to 63.4%.

Table 2. Plasma levels of biogenic amines during short-term neuroleptic treatment

	Typical neuroleptics n = 15	Clozapine n = 15	Statistics Wilcoxon signed rank test
MHPG (ng/ml)	0.84 ± 0.55	0.88 ± 0.55	$p = 0.583$
Norepinephrine (pg/ml)	333.53 ± 230.50	733.81 ± 336.82	$p = 0.0006$***
Epinephrine (pg/ml)	162.90 ± 149.55	286.48 ± 219.89	$p = 0.083$
Dopamine (pg/ml)	75.36 ± 107.79	45.04 ± 27.17	$p = 0.934$
Serotonin (ng/ml)	191.19 ± 109.01	234.94 ± 127.57	$p = 0.029$*

Individual values (mean ± SD) were calculated from two consecutive measurements at weeks 5 and 6 (typical neuroleptic treatment) and at weeks 11 and 12 (clozapine treatment). * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$

For the comparison between responders and nonresponders to clozapine, individual mean values of two measurements at weeks 5 and 6 during typical neuroleptic treatment and at weeks 5 and 6 of clozapine administration were calculated. Responders to clozapine showed an overall improvement as revealed by ratings of both positive (Median test DF=1; $\chi^2 = 5.16$; $p = 0.023$*) and negative symptoms (t-Test unequal DF=12.4, T=2.72; $p = 0.018$*) as well as BPRS-derived depressive (Median test DF=1, $\chi^2 = 8.17$; $p = 0.004$**) and BPRS total score symptoms ($\chi^2 = 5.16$; $p = 0.02$*). Responders to clozapine differed from nonresponders by significant lower depressive symptoms during pretreatment with typical neuroleptics (Median test DF=1; $\chi^2 = 5.16$; $p = 0.02$*). However, the distribution of both positive and negative symptoms before-clozapine demonstrated no significant differences in responders and nonresponders to clozapine.

A comparison of individual mean plasma epinephrine levels in responders and nonresponders to clozapine revealed that response to clozapine can be predicted by epinephrine levels prior to initiation of treatment with clozapine at weeks 5 and 6 (cut-off: 91 pg/ml): responders ranging from 32.2 to 90.3 pg/ml and nonresponders ranging from 92.5 to 473.5 pg/ml (Table 3).

During clozapine treatment, plasma concentrations of MHPG ($p = 0.031$) and epinephrine ($p = 0.031$) of responders increased as compared to pretreat-

Table 3. Plasma levels of epinephrine in responders (n = 7) and nonresponders (n = 8) to clozapine during pretreatment with typical neuroleptics at weeks 5 and 6

	Epinephrine plasma levels [pg/ml]		Median test DF = 1	
Week	Responder	Nonresponder	χ^2	p
5 Typical neuroleptics	77.39 ± 47.40	247.82 ± 221.35	5.16	0.02*
6 Typical neuroleptics	41.91 ± 24.85	227.85 ± 196.81	7.86	0.005**

* = $p \leq 0.05$, ** = $p \leq 0.01$

ment levels. Nonresponders to clozapine failed to show this increase in the plasma concentrations of MHPG and epinephrine. The concentrations of plasma norepinephrine and serum serotonin did not differentiate between responders and nonresponders to clozapine. However, patients who responded to clozapine showed a negative linear relationship (Spearman correlation) between negative symptoms (SANS) of schizophrenia and the concentrations of plasma norepinephrine ($r = -0.81$; $p = 0.027$) and serum serotonin ($r = -0.93$; $p = 0.008$), respectively. This finding implies that during short-term treatment with clozapine, increasing levels of plasma norepinephrine or serum serotonin predict an improvement in negative symptom scores of schizophrenia.

Analyses of the relationships between clozapine dose and serum levels of clozapine and its metabolites were carried out under steady state conditions at weeks 5 and 6 of clozapine treatment. At that time, the 15 patients were treated with oral doses of clozapine varying from 100 to 600 mg daily. Patients were compliant with clozapine medication as evidenced by serum level monitoring.

At weeks 5 and 6 of clozapine treatment, we found a linear relationship (Spearman correlation) between doses ranging from 100 to 600 mg daily and the measured serum levels of clozapine ($r = 0.78$; $p = 0.001$), norclozapine ($r = 0.72$; $p = 0.003$) and clozapine N-oxide ($r = 0.53$; $p = 0.05$), respectively. The mean ± SD ratio of norclozapine/clozapine was 1.39 ± 0.49, and the ratio of clozapine N-oxide/clozapine was 0.48 ± 0.27. The norclozapine and clozapine N-oxide metabolites achieved concentrations ranging from 78% to 240% and 11% to 98%, respectively, of those of the parent drug.

Discussion

In agreement with our previous report including 40 adolescent patients with a one-year follow-up (Schulz et al., 1996), this six-week study confirmed that over the course of treatment the serum levels of serotonin increased during clozapine medication as compared to pretreatment levels. Banki (1978) first described elevated total blood serotonin concentrations due to clozapine treatment in adults. This finding was replicated by our group investigating the short- and long-term effects of clozapine on plasma biogenic amines in adolescents with schizophrenia.

Our ongoing prospective and open clinical trial shows that clozapine acts on both norepinephrine and serotonin in a rapid manner resulting in a long-lasting increase during follow-up observation. In addition, responders to clozapine were characterised by increasing plasma levels of MHPG and epinephrine, whereas nonresponders failed to show any significant changes.

Assessment of psychopathology and biogenic amines before-clozapine revealed that only ratings of low depressive symptomatology and epinephrine plasma concentrations below a cut-off of 91 pg/ml could be looked upon as predictors of a positive response to clozapine treatment. These findings need further elucidation.

The finding of markedly increased plasma norepinephrine levels is in agreement with our results on long-term clozapine treatment (Schulz et al., 1996) and

concurs with studies in adult patients, which report clozapine-induced increases in plasma and/or CSF levels of norepinephrine in adults with schizophrenia (Breier et al., 1994; Green et al., 1993; Lieberman et al., 1991; Pickar et al., 1992). Breier et al. (1994) reported that clozapine produced a significant increase in plasma norepinephrine levels and that this increase was positively correlated with a reduction in symptoms as assessed with BPRS total scores and positive symptom scores. Typical neuroleptic drugs failed to show this relationship.

Green et al. (1993) were able to demonstrate that plasma norepinephrine levels increased in both responders and nonresponders to clozapine. This finding is in agreement with our results. Interestingly, we were able to demonstrate, that patients who became responders to clozapine showed a negative linear relationship between negative symptoms of schizophrenia and the concentrations of plasma norepinephrine and of serum serotonin levels.

No change was found so long for CSF serotonin or 5-hydroxyindoleacetic acid concentrations in clozapine treated patients, though serum serotonin levels were found to be significantly enhanced. The activity of the serotoninergic neurons, especially in the nucleus raphe dorsalis, major source of serotoninergic neurons in the brain, seems to be enhanced, as was shown by measures of neuron stimulation through secretogranin II and chromogranin A mRNA (Kroesen et al., 1995). But this might be counteracted by the increased activity of the serotonin transporter (Naylor et al., 1996). The increased serotonin level in serum might therefore also be due to an enhanced activity of the serotonin transporter in platelets, which is structurally identical with the brain serotonin transporter (Lesch et al., 1993). Since in schizophrenics mainly the affinity of the transporter is affected, the platelet serotonin transporter might be a target to study changes in brain serotonin transporter modifications.

The observed elevated plasma levels of norepinephrine after clozapine treatment might correlate with CSF levels of norepinephrine, since this was indicated in studies on adult schizophrenics (Ackenheil, 1989; Breier et al., 1994; Green et al., 1993; Lieberman et al., 1991; Pickar et al., 1992; Sarafoff et al., 1979). Increased CSF and plasma levels of norepinephrine might reflect a general increase in the activity of the brain noradrenergic and sympathetic nervous system, leading also to the observed changes in plasma epinephrine levels. Despite the ongoing uncertainty of plasma MHPG that derives from the brain, it is clear that plasma measurements of MHPG reflect norepinephrine metabolism and are continuing to be pursued as clinical research tool (Green et al., 1993).

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References

Ackenheil M (1989) Clozapine: pharmacokinetic investigations and biochemical effects in man. Psychopharmacol 99: 32–37

American Psychiatric Association (1987) Diagnostic and statistical manual of mental disorders, 3rd edn. American Psychiatric Association, Washington

Andreasen NC (1982) Negative symptoms in schizophrenia: definition and reliability. Arch Gen Psychiatry 39: 784–788

Andreasen NC (1984a) The Scale for the Assessment of Negative Symptoms (SANS). University of Iowa, Iowa City

Andreasen NC (1984b) The Scale for the Assessment of Positive Symptoms (SAPS). University of Iowa, Iowa City

Andreasen NC, Flaum M, Arndt S, Alliger R, Swayze VW (1991) Positive and negative symptoms: assessment and validity. In: Marneros A, Andreasen NC, Tsuang MT (eds) Negative versus positive schizophrenia. Springer, Berlin Heidelberg New York Tokyo, pp 28–51

Arora RC, Meltzer HY (1991) Serotonin$_2$ (5-HT$_2$) receptor binding in the frontal cortex of schizophrenic patients. J Neural Transm [Gen Sect] 85: 19–29

Baldessarini RJ, Huston-Lyons D, Campbell A, Marsh E, Cohen BM (1992) Do central antiadrenergic actions contribute to the atypical properties of clozapine? Br J Psychiatry [Suppl]: 12–16

Banki CM (1978) Alterations of cerebrospinal fluid 5-hydroxyindoleacetic acid and total blood serotonin content during clozapine treatment. Psychopharmacol 56: 195–198

Birmaher B, Baker R, Kapur S, Quintana H, Ganguli R (1992) Clozapine for the treatment of adolescents with schizophrenia. J Am Acad Child Adolesc Psychiat 31: 160–164

Blanz B, Schmidt MH (1993) Clozapine for schizophrenia. J Am Acad Child Adolesc Psychiat 32: 223–224

Boomsma F, Alberts G, Vaneijk L, Manintveld AJ, Schalekamp MADH (1993) Optimal collection and storage conditions for catecholamine measurements in human plasma and urine. Clin Chem 39: 2503–2508

Breier A (1995) Serotonin, schizophrenia and antipsychotic drug action. Schizophr Res 14: 187–202

Breier A, Buchanan RW, Waltrip RW, Listwak S, Holmes G, Goldstein DS (1994) The effect of clozapine on plasma norepinephrine: relationship to clinical efficacy. Neuropsychopharmacol 10: 1–7

Brunello N, Masotto, C, Steardo L, Markstein R, Racagni G (1995) New insights into the biology of schizophrenia through the mechanism of action of clozapine. Neuropsychopharmacol 13: 177-213

Davis KL, Kahn RS, Ko G, Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. Am J Psychiatry 148: 1474–1486

Frazier JA, Gordon CT, McKenna K, Lenane MC, Jih D, Rapoport JL (1994) An open trial of clozapine in 11 adolescents with childhood-onset schizophrenia. J Am Acad Child Adolesc Psychiat 33: 658–663

Ganhao MF, Hattingh J, Hurwitz ML, Pitts NI (1991) Evaluation of a simple plasma catecholamine extraction procedure prior to high-performance liquid chromatography and electrochemical detection. J Chromatogr 564: 55–66

Gordon CT, Frazier JA, McKenna K, Giedd J, Zametkin A, Zahn T, Hommer D, Hong W, Kaysen D, Albus KE, Rapoport JL (1994) Childhood-onset schizophrenia: an NIMH study in progress. Schizophr Bull 20: 697–712

Green AI, Alam MY, Sobieraj JT, Pappalardo KM, Waternaux C, Salzman C, Schatzberg AF, Schildkraut JJ (1993) Clozapine response and plasma catecholamines and their metabolites. Psychiat Res 46: 139–149

Kahn RS, Davidson M (1993) On the value of measuring dopamine, norepinephrine and their metabolites in schizophrenia. Neuropsychopharmacol 8: 93–95

Kapur S, Remington G (1996) Serotonin-dopamine interaction and ist relevance to schizophrenia. Am J Psychiatry 153: 466–476

Kroesen S, Marksteiner J, Mahata M, Fischer Colbrie R, Saria A, Kapeller I, Winkler H (1995) Effects of haloperidol, clozapine and citalopram on messenger RNA levels of chromogranins A and B and secretogranin II in various regions of rat brain. Neurosci 69: 881–891

Kronig MH, Munne RA, Szymanski S, Safferman AZ, Pollack S, Cooper T, Kane JM, Lieberman JA (1995) Plasma clozapine levels and clinical response for treatment-refractory schizophrenic patients. Am J Psychiatry 152: 179–182

Lake CR, Ziegler MG (1985) Techniques for the assessment and interpretation of catecholamine measurements in neuropsychiatric patients. In: Lake CR, Ziegler MG (eds) The catecholamines in psychiatric and neurologic disorders. Butterworth Publishers, Stone-Hane, pp 1–34

Lesch KP, Wolzin BL, Murphy DL, Riederer P (1993) Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. J Neurochem 60: 2319–2322

Lieberman JA, John C, Pollack S, Masier S, Bookstein P, Cooper T, Iadorola M, Kane J (1991) Biochemical effects of clozapine in cerebrospinal fluid of patients with schizophrenia. In: Tamminga CA, Schulz S (eds) Schizophrenia research, vol 1. Raven Press, New York, pp 341–349

Meszaros K, Lenzinger E, Fureder T, Hornik K, Willinger U, Stompe T, Heiden AM, Resinger E, Fathi N, Gerhard E, Fuchs K, Miller-Reiter E, Pfersmann V, Sieghart W, Aschauer HN, Kasper S (1996) Schizophrenia and the dopamine beta hydroxylase gene: results of a linkage and association study. Psychiatr Genet 6: 17–22

Miller DD, Fleming F, Holman T, Perry PJ (1994) Plasma clozapine concentrations as a predictor of clinical response: a follow-up study. J Clin Psychiat 55: 117–121

Moscarelli M, Maffei C, Cesana BM, Boato B (1987) An international perspective on assessment of negative and positive symptoms in schizophrenia. Am J Psychiatry 144: 1595–1598

Mozes T, Toren P, Chernauzan N, Mester R, Yoranhegesh R, Blumensohn R, Weizman A (1994) Clozapine treatment in very early onset schizophrenia. J Am Acad Child Adolesc Psychiat 33: 65–70

Naylor L, Dean B, Opeskin K, Pavey G, Hill C, Keks N, Copolov D (1996) Changes in the serotonin transporter in the hippocampus of subjects with schizophrenia identified using [^{3}H]paroxetine. J Neural Transm 103: 749–757

Orellana-Vidal GP (1995) Locus coeruleus: implications for psychiatry. Acta Psiquiat Psicol Am Lat 41: 57–66

Overall JE, Gorham DR (1962) The Brief Psychiatric Rating Scale. Psychol Rep 10: 799–812

Pickar D, Owen RR, Litman RE, Konicki PE, Gutierrez R, Rapaport MH (1992) Clinical and biologic response to clozapine in patients with schizophrenia: crossover comparison with fluphenazine. Arch Gen Psychiatry 49: 345–353

Piscitelli SC, Frazier JA, McKenna K, Albus KE, Grothe DR, Gordon CT, Rapoport JL (1994) Plasma clozapine and haloperidol concentrations in adolescents with childhood-onset schizophrenia–association with response. J Clin Psychiatry 55: 94–97

Pluto R, Bürger P (1988) Normal values of catecholamines in blood plasma determined by high-performance liquid chromatography with amperometric detection. Int J Sports Med 9: 75–78

Potkin SG, Bera R, Gulasekaram B, Costa J, Hayes S, Jin Y, Richmond G, Carreon D, Sitanggan K, Gerber B (1994) Plasma clozapine concentrations predict clinical response in treatment-resistant schizophrenia. J Clin Psychiatry 55: 133–136

Remschmidt H, Martin M, Schulz E, Gutenbrunner C, Fleischhaker C (1991) The concept of positive and negative schizophrenia in child and adolescent psychiatry. In: Marneros A, Andreasen NC, Tsuang MT (eds) Negative versus positive schizophrenia. Springer, Berlin Heidelberg New York Tokyo, pp 219–242

Remschmidt H, Schulz E, Martin M (1992) Die Behandlung schizophrener Psychosen in der Adoleszenz mit Clozapine (Leponex). In: Naber D, Müller-Spahn F (Hrsg) Clozapine. Pharmakologie und Klinik eines atypischen Neuroleptikums. Schattauer, New York, S 99–119

Remschmidt H, Schulz E, Martin M (1994a) An open trial of clozapine in thirty-six adolescents with schizophrenia. J Child Adolesc Psychopharmacol 4: 31–41

Remschmidt H, Schulz E, Martin M, Warnke A, Trott GE (1994b) Childhood-onset schizophrenia: history of the concept and recent studies. Schizophr Bull 20: 727–745

Riederer P, Laux G (1992) Therapeutic drug monitoring of psychotropics: report of a consensus conference. Pharmacopsychiat 25: 271–272

Schulz E, Fleischhaker C, Remschmidt H (1995) Determination of clozapine and its major metabolites in serum samples of adolescent schizophrenic patients by high performance liquid chromatography. Data from a prospective clinical trial. Pharmacopsychiat 28: 20–25

Schulz E, Fleischhaker C, Remschmidt H (1996) Correlated changes in symptoms and neurotransmitter indices during maintenance treatment with clozapine or conventional neuroleptics in adolescents and young adults with schizophrenia. J Child Adolesc Psychopharmacol 6: 119–131

Seeger TF, Seymour PA, Schmidt AW, Zorn SH, Schulz DW, Lebel LA, McLean S, Guanowsky V, Howard HR, Lowe JA (1995) Ziprasidone (CP-88,059): a new antipsychotic with combined dopamine and serotonin receptor antagonist activity. J Pharmacol Exp Ther 275: 101–113

Siefen G, Remschmidt H (1986) Behandlungsergebnisse mit Clozapine bei schizophrenen Jugendlichen. Z Kinder Jugendpsychiat 14: 245–257

Towbin KE, Dykens EM, Pugliese RG (1994) Clozapine for early developmental delays with childhood-onset schizophrenia – protocol and 15-month outcome. J Am Acad Child Adolesc Psychiat 33: 651–657

van Kammen DP (1991) The biochemical basis of relaps and drug response in schizophrenia: review and hypothesis. Psychol Med 21: 881–895

van Kammen DP, Kelley M (1991) Dopamine and norepinephrine activity in schizophrenia. An integrative perspective. Schizophr Res 4: 173–191

Weier TB, Smith CT, Round JM, Betteridge DJ (1986) Stability of catecholamines in whole blood, plasma, and platelets. Clin Chem 32: 822–833

Yamamoto K, Ozawa N, Shinba T, Hoshino T, Yoshii M (1994) Possible noradrenergic dysfunction in schizophrenia. Brain Res Bull 35: 529–543

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