Effect of Clozapine Treatment on Serotonin-2-Receptor Binding in the Blood Platelets of Schizophrenic Patients

Ramesh C. Arora, Ph.D.[†] and Herbert Y. Meltzer, M.D.

The effect of clozapine and typical neuroleptic drug treatment on platelet serotonin₂ (5-HT₂) binding kinetic constants (K_d , B_{max}) was studied in schizophrenic patients. Both treatments increased B_{max} by a comparable amount, indicating increased numbers of 5-HT₂ sites, although not all typical neuroleptic drugs were effective in this regard. Clozapine, but not typical neuroleptic

drugs, increased K_d , indicating a lower affinity of the 5-HT₂ sites for 5-HT. In a multiple regression model, low B_{max} at baseline predicted poor outcome on Brief Psychiatric Rating Scale measures in the clozapine-treated patients but not the neuroleptic-treated patients. [Neuropsychopharmacology 10:109–114, 1994]

KEY WORDS: Clozapine; Serotonin; Schizophrenia; Neuroleptic treatment; Platelet receptor binding

Clozapine has been shown to be an effective antipsychotic drug for the treatment of neuroleptic-resistant schizophrenic patients and to produce fewer extrapyramidal symptoms (Kane et al. 1988; Meltzer 1990). It has been suggested that the ability of clozapine to block central serotonin-2 (5-HT₂) receptors may contribute to its clinical advantages (see Meltzer 1989, 1991, for reviews).

In vivo, clozapine has been reported to downregulate 5-HT₂ receptor in rat frontal cortex after acute (Matsubara and Meltzer 1989) and chronic treatment (Reynolds et al., 1983; Lee and Tang 1984; Ellis et al. 1988; Matsubara and Meltzer 1978). This effect has been attributed to its 5-HT₂ antagonist properties.

From the Laboratory of Biological Psychiatry, Department of Psychiatry, School of Medicine, Case Western Reserve University, Cleveland, Ohio.

Address correspondence to: Herbert Y. Meltzer, M.D., Department of Psychiatry, School of Medicine, Case Western Reserve University, 2040 Abington Road, Cleveland, OH 44106-5000.

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Serotonin-2 receptor binding has been studied in the postmortem brains of schizophrenic patients. However, the results are controversial. Increased (Whitaker et al. 1981), decreased (Bennett et al. 1979; Mita et al. 1986; Arora and Meltzer 1991; Laruelle et al. 1991) or normal (Mackay et al. 1978; Owen et al. 1981; Reynolds et al. 1983) 5-HT₂ binding has been reported in the frontal cortex of neuroleptic-treated schizophrenic patients. Because neuroleptic drugs may downregulate 5-HT₂ receptors in rodent brain (Andree et al. 1986; Lee and Tang 1984), these results could be influenced by neuroleptic treatment. However, Mita et al. (1986) and Laruelle et al. (1991) did not find any differences between the 5-HT₂ binding in the frontal cortex of neuroleptic-treated and unmedicated schizophrenic patients. Consistent with the concept of downregulated 5-HT₂ receptors in schizophrenia, Lee et al. (1992) reported that the hypothermic response following administration of MK-212, a centrally-acting 5-HT_{1C}/5-HT₂ agonist, was decreased in schizophrenic patients.

A 5-HT₂ receptor with similar pharmacological properties to that of human brain has been identified in human blood platelets (Geaney et al. 1984; Elliott and Kent 1989). Increased 5-HT₂ binding was found in the blood platelets of suicidal compared to non-suicidal

schizophrenic patients and normal controls (Arora and Meltzer 1993). No significant group difference was observed between schizophrenic patients and normal controls (Arora and Meltzer 1993). No significant group difference was observed between schizophrenic patients and normal controls (Arora and Meltzer 1993). Schachter et al. (1985) reported increased K_d and B_{max} of 5-HT₂ binding in the blood platelets of neuroleptic-(fluphenazine, flupenthixol, clopenthixol, chlorpromazine)-treated schizophrenic patients. We have now studied the effect of chronic clozapine treatment on 5-HT₂ receptor binding in the blood platelets of schizophrenic patients. In addition, we studied the effect of treatment with typical neuroleptic drugs on platelet 5-HT₂ receptor binding and compared the results with those from clozapine-treated schizophrenic patients.

METHODS

All patients were diagnosed according to Research Diagnostic Criteria (RDC) (Spitzer et al. 1978) with data obtained with the Schedule for Affective Disorders and Schizophrenia (Endicott and Spitzer 1978) and all information available from the entire hospital course. Twenty-nine patients (21 male, eight female) had not received any psychotic drugs for at least seven days before study. Twenty-five (20 male, five female) clozapine-treated patients ($\bar{x}$ SD = 467 $\pm$ 132 mg/day, range 125 mg to 900 mg/day) were studied: 20 between 12 and 60 days of treatment, one after 76 days, three after 150 to 200 days, and one for 694 days. Thirty-two (26 male, six female) schizophrenic patients treated with typical neuroleptic drugs were studied. The duration of treatment in 30 patients was 15 to 70 days; one was studied after 240 days and another after 365 days of treatment. The antipsychotic drugs utilized were haloperidol (n = 9), thioridazine (n = 7), trifluoperazine (n = 3), loxapine (n = 4), perphenazine (n = 3) and fluphenazine (n = 6). The mean dose in chlorpromazine equivalents was 540 $\pm$ 113 mg/day.

The mean age of unmedicated, clozapine-treated, and typical neuroleptic-treated patients was 36.1 $\pm$ 8.3, 34.5 $\pm$ 8.9, and 34.6 $\pm$ 10.0 years, respectively. There were no differences in age between the three groups.

All results are mean $\pm$ SD.

Determination of Platelet 5-HT₂ Receptor Binding

Blood (30 ml) was drawn into plastic syringes and transferred into plastic tubes containing 1% ethylenediaminetetraacetic acid in saline as anticoagulant (1 ml/9 ml of blood). Platelet-rich plasma was obtained by centrifugation at 600 $\times$ g for 2.5 minutes in a Sorvall GLC-2

centrifuge using a swinging bucket rotor as described earlier (Sahai et al. 1981). Platelet membranes were prepared following the method of Geaney et al. (1984).

Serotonin-2 receptor binding was determined by incubating platelet membranes with ³H-lysergic acid diethylamide (³H-LSD) as described earlier (Arora and Meltzer 1989). Five concentrations of ³H-LSD between 0.2 nmol/L to 4.0 nmol/L were used in the incubation mixture to determine the apparent dissociation constant (K_d) and the maximum number of specific binding sites (B_{max}) by Scatchard analysis. Protein in the platelet membrane preparation was determined by Lowry's method (Lowry et al. 1951). The K_d and B_{max} in 42 normal controls were 0.72 $\pm$ 0.31 (SD) nM and 84.6 $\pm$ 24.9 fmol/ng protein (Arora and Meltzer 1993).

The radioactive ³H-LSD (sp. act. 79.9 Ci/mmol) was purchased from New England Nuclear (Boston, MA). All of the other chemicals used were of analytical grade quality.

RESULTS

The kinetic parameters (K_d and B_{max}) of ³H-LSD binding in the blood platelets of unmedicated schizophrenic patients during clozapine treatment or typical neuroleptic treatment are reported in Table 1. There were no significant differences between B_{max} and K_d of the neuroleptic-withdrawn schizophrenic patients and the normal controls. The correlations between number of days washout and B_{max} or K_d were not significant (r = 0.25 and r = 0.09, respectively). There were three subjects with washouts of 20 or more days. There was no evidence for lower B_{max} levels in this group compared to nine patients with washouts of only 7 to 10 days (118 $\pm$ 9 versus 90 $\pm$ 16 fmol/mg protein). Correlations between K_d and B_{max} , and the number of days of treatment, or the doses of clozapine or typical neuroleptic drugs in chlorpromazine equivalents, were not significant (data not presented). Hence, the data from different days and different doses were combined and reported in Table 1. Univariate analyses of variance indicated significant group difference in K_d and B_{max} of ³H-LSD binding. B_{max} was significantly increased in the clozapine- and typical neuroleptic-treated patients compared to the drug-free patients. However, there was no difference in B_{max} between the two drug-treated groups. K_d was also significantly increased during clozapine treatment but not during typical neuroleptic treatment. Further analysis of individual drug treatment in the typical neuroleptic group indicated that a significant increase in B_{max} was observed only in the thioridazine- (n = 7), loxapine- (n = 4) and fluphenazine- (n = 6) treated patients. There was a trend for increase

Table 1. Effect of Clozapine and Typical Neuroleptics on ^3H -LSD Binding in the Blood Platelets of Schizophrenic Patients

Treatment	n	K_d (nmol/L)	$B_{\max}$ (fmols/mg/protein)
No treatment	29	0.68 ± 0.32	97.8 ± 30.07
Clozapine	25	$1.19 \pm 0.64^*$	$177.6 \pm 70.0^*$
Typical neuroleptics	32	0.80 ± 0.36	$140.4 \pm 52.0^*$
Haloperidol	9	0.71 ± 0.42	$117.7 \pm 41.4^{**}$
Thioridazine	7	0.85 ± 0.23	$172.6 \pm 54.8^*$
Fluphenazine	6	0.89 ± 0.52	$151.7 \pm 27.6^*$
Loxapine	4	0.72 ± 0.20	$181.8 \pm 69.2^*$
Perphenazine	3	0.97 ± 0.49	98.4 ± 14.5
Trifluoperazine	3	0.67 ± 0.25	97.9 ± 39.9

* Significantly higher than no treatment group.

** $t = 1.57$; $p < 0.05 > 0.1$.

Data are expressed as $\pm$ SD.

in $B_{\max}$ in the haloperidol-treated patients ($n = 9$; $p < .1 > .05$). There was no suggestion of a difference between untreated and trifluoperazine ($n = 3$)- and perphenazine ($n = 3$)-treated patients.

We also studied 11 patients pre- and post-clozapine treatment and nine patients pre- and post-typical neuroleptic treatment (thioridazine, $n = 4$; loxapine, $n = 2$; haloperidol, trifluoperazine and perphenazine, each one patient). The mean duration of treatment was 39.6 ± 14.2 days. The mean dose of neuroleptic in chlorpromazine equivalents was 643 ± 169 mg/day. The results are reported in Fig. 1 and Fig. 2, respectively. As evident from Figure 1, we again observed significant increases in K_d and $B_{\max}$ following clozapine treatment. Similarly, $B_{\max}$, but not K_d , increased during treatment with typical neuroleptic drugs (Fig. 2).

There were no significant correlations between change in $B_{\max}$ or K_d and changes in BPRS Total and Positive symptoms at 6 weeks. However, there was a

trend for a significant positive correlation between change in $B_{\max}$ and change in negative symptoms ($\rho = .585$; $p = .058$) after 6 weeks of clozapine treatment. There were also significant negative correlations between $\Delta B_{\max}$ and Δ BPRS Total ($\rho = -0.79$; $n = 7$; $p = .04$) and Δ BPRS Positive ($\rho = -0.81$; $n = 7$, $p = .03$) for the typical neuroleptic-treated patients.

In a multiple regression model in which BPRS Total at 6 weeks of clozapine treatment was predicted from Baseline BPRS Total, pretreatment K_d and $B_{\max}$, $B_{\max}$ (standard estimate = -0.60 , $p = .01$, $df = 1.10$) significantly predicted BPRS Total at 6 weeks. Pretreatment $B_{\max}$ also was inversely related to outcome as indicated by BPRS Positive Symptoms ($p = .005$). There were no significant relationships between post-treatment BPRS negative symptoms and $B_{\max}$ or K_d for the clozapine-treated patients in the multivariate model. Similarly, there were no relationships between pretreatment or post-treatment $B_{\max}$ or K_d and BPRS Total, Positive, or Negative symptoms in the neuroleptic-treated patients.

DISCUSSION

The major finding in this study is that clozapine treatment increased K_d and $B_{\max}$ of 5-HT₂ receptor binding in the blood platelets of schizophrenic patients, whereas typical neuroleptic treatment increased only the $B_{\max}$ of 5-HT₂ binding. No difference in K_d was observed between pre- and post-typical neuroleptic treatment. There was a nearly significant positive correlation ($p = .058$) between the change in $B_{\max}$ during clozapine treatment and the change in negative symptoms of schizophrenic patients. Furthermore, there was also a suggestion that low baseline $B_{\max}$ predicts a poor response to clozapine and that the increase in $B_{\max}$ dur-

Figure 1. Effect of clozapine treatment on K_d and $B_{\max}$ of ^3H -LSD binding in the blood platelets of 11 schizophrenic patients. K_d increased from $.63 \pm .22$ nM to $1.27 \pm .43$ ($t = 4.45$; $p < .005$; paired t -test). $B_{\max}$ increased from 89.5 ± 17.3 fmol/mg protein to 175.5 ± 60.6 fmol/mg protein ($t = 4.78$; $p < .005$; paired t -test).

Figure 2. Effect of typical neuroleptic treatment on K_d and B_{max} of 3H -LSD binding in the blood platelets of nine schizophrenic patients. K_d did not significantly change ($.86 \pm \text{nmol/L}$ prior to treatment versus $1.02 \pm .41 \text{ nmol/L}$; $t = 1.21$; $p = \text{NS}$; paired t -test). B_{max} increased from $97.9 \pm 40.0 \text{ fmol/mg protein}$ to $142.2 \pm 65.8 \text{ fmol/mg protein}$ ($t = 2.93$; $p < .02$; paired t -test).

ing neuroleptic treatment was associated with less improvement.

The possibility that the washout from prior neuroleptic treatment was inadequate was considered. The mean duration was 12.0 days, which is approximately two half-lives of human blood platelets, so that fully 75% of the platelets should have been replaced at the time of study. Had there been a residual effect of prior neuroleptic treatment, an increase in B_{max} compared to normal controls would have been found, but this was not observed. We also found no correlation between B_{max} or K_d and the duration of washout. Therefore, we believe the results reported here are not significantly affected by prior neuroleptic treatment.

The increase in B_{max} in platelets during clozapine treatment and treatment with some neuroleptic drugs is contrary to what has been observed in brain (see below) with these drugs but is consistent with the usual effect of antagonists to upregulate the number of binding sites.

The K_d and B_{max} values of platelet 5-HT₂ receptor binding in normal controls and schizophrenic patients reported here are similar to those reported earlier in another sample (Arora and Meltzer 1989; 1993). Increased 5-HT₂ binding following neuroleptic treatment (present study) is consistent with the observations of increased platelet function believed to be mediated by 5-HT₂ receptors, (i.e., platelet aggregation response to 5-HT₂ during chlorpromazine treatment [Oppenheim et al. 1980]) and chlorpromazine, fluphenazine, flupenthixol, trifluoperazine, or haloperidol treatment of schizophrenic patients (Orr et al. 1981). A significant correlation has been previously reported between 5-HT₂ receptor binding in human blood platelets and the 5-HT-amplified aggregation response (McBride et al. 1987). Animal studies also indicated increased platelet 5-HT₂ receptor function following chlorpromazine treatment as evidenced by increased aggregation re-

sponse to 5-HT in the blood platelets of chlorpromazine-treated rabbits (Baldacci et al. 1980). Increased 5-HT₂ binding has also been reported in rat blood platelets following chronic treatment with ritanserin, which like clozapine is a potent 5-HT₂ receptor antagonist (Twist et al. 1990). Although these results with ritanserin would suggest that blockade of 5-HT₂ receptor accounts for the upregulation of the platelet 5-HT₂ binding sites, some of the typical neuroleptic drugs that cause upregulation have a low affinity for 5-HT₂ receptors (e.g., fluphenazine and thioridazine [Meltzer et al., 1989]). Thus, the biological basis for the changes in B_{max} remain obscure.

The results reported here with typical neuroleptic drugs are in partial agreement with those reported by Schachter et al. (1985) who reported an increase in K_d as well as B_{max} of 5-HT₂ receptor binding in schizophrenic patients receiving depot neuroleptic treatment. Schachter et al. (1985) speculated that neuroleptic-induced increases in K_d may be due to drug retention during preparation of platelet membranes. The procedure of Geaney et al. (1984) for preparing platelet membranes was utilized both in this study and in that of Schachter et al. (1985). Furthermore, platelet membranes were prepared the same way for both typical and atypical (clozapine)-antipsychotic drug treated patients. Because relatively small numbers of subjects were studied with each of the typical neuroleptic drugs, it is not possible to draw firm conclusions about the presence of qualitative differences among them. It would appear, however, that the high potency drugs such as haloperidol, fluphenazine, perphenazine, and trifluoperazine, are less effective than the low potency neuroleptic, thioridazine, or the clozapine analog, loxapine with regard to increasing B_{max} . The minimal effects of the high potency agents on B_{max} or K_d suggests that the D₂ dopamine receptor blocking effect of these agents is not important to this effect. Because loxapine,

a potent 5-HT₂ antagonist (Meltzer et al. 1989), did not appear to increase K_d , the ability of clozapine to increase K_d is not necessarily related to its affinity for 5-HT₂ receptors.

The effects of typical neuroleptics on 5-HT₂ binding in rodent brain are controversial. Wilmot and Szczepanik (1989) and others (Lee and Tang 1984; Peroutka and Snyder 1980) did not report any effect of chronic haloperidol or chlorpromazine treatment on 5-HT₂ binding in rat brain, whereas Lee and Tang (1984) and Andree et al. (1986) reported significantly decreased 5-HT₂ binding in rat frontal cortex following chronic neuroleptic treatment with chlorpromazine, thioridazine, loxapine, or clozapine. The extent of the decrease in B_{max} produced by different neuroleptics was variable; chlorpromazine (Andree et al. 1986) produced the strongest effect. Clozapine administration has also been reported to rapidly and markedly downregulate 5-HT₂ receptors in rat frontal cortex (Matsubara and Meltzer 1989).

Human studies also indicated no significant difference in 5-HT₂ binding in the frontal cortex of neuroleptic-treated and drug-free schizophrenic patients (Mita et al. 1986). We (Arora and Meltzer 1991) studied 5-HT₂ binding in the frontal cortex of neuroleptic-treated schizophrenic patients and could find no evidence that neuroleptics downregulate 5-HT₂ binding in humans. These apparent differences in the effect of neuroleptic and clozapine treatment in platelet 5-HT₂ binding and animal brain 5-HT₂ binding studies suggests that there are different regulatory mechanisms governing platelet and neuronal 5-HT₂ receptors and that blood platelets may not be a good model for the effect of antipsychotic drugs on brain 5-HT₂ receptors.

Similar discrepancies between platelet and brain 5-HT₂ binding sites have been reported with tricyclic antidepressants. They have been shown to downregulate 5-HT₂ receptors in rat brain in some but not all studies (see Twist et al. 1990; Willner 1985 for review). Increased 5-HT₂ binding has been reported in blood platelets in normal volunteers following chronic desipramine treatment (Cowen et al. 1986), whereas decreased 5-HT₂ binding has been reported in the blood platelets of depressed patients following tricyclic antidepressant treatment (Biegon et al. 1987). There is no evidence that clozapine or typical neuroleptic drugs lead to increased 5-HT₂ receptor-mediated activity because the cortisol response to 5-hydroxytryptophan (5-HT) is blocked by clozapine treatment (Meltzer, H. Y., in preparation).

The finding that low B_{max} predicted poor response to clozapine at 6 weeks is intriguing but must be verified in an independent sample. If this is a reliable finding, it might be helpful in identifying good candidates for clozapine the increase in platelet 5-HT₂ binding sites reflects a

similar increase in brain 5-HT₂ binding sites for clozapine and some typical neuroleptic drugs. Similar considerations apply to the increase in platelet K_d and possible effect of clozapine on the K_d of the brain 5-HT₂ receptors.

Further studies of the function of the 5-HT₂ receptor in clozapine-treated or typical neuroleptic-treated patients are indicated to determine if the results reported here influence the effects of 5-HT on platelet shape change or platelet aggregation. To our knowledge, no platelet-related abnormalities have been reported in patients treated with these drugs that could be ascribed to changes in B_{max} or K_d , or both.

In summary, treatment of schizophrenic patients with clozapine or some typical neuroleptic drugs decreases the number of 5-HT₂ sites in blood platelets. These results are contrary to what has been observed in rodent brain and may also differ from the effects of these drugs on brain 5-HT₂ receptors during clinical treatment. However, there was some evidence for the ability of baseline 5-HT₂ binding sites (B_{max}) to predict response to clozapine. Because of the small number of patients in this study ($n = 11$), further study with more patients is needed to confirm these results.

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NOTE ADDED IN PROOF

Two recent studies have found that the binding characteristics of the 5-HT₂ receptor in main cortex synaptosomes and platelet membranes in man (Andres et al. 1993) and pigs (Ostrowitzki et al. 1993) were correlated and were suggested to be similarly regulated: Andres AH, Rao ML, Ostrowitzki S, and Entzian W (1993): Human brain cortex and platelet serotonin-2-receptor binding properties and their regulation by endogenous serotonin. *Life Sci* 52:313-321. Ostrowitzki S, Rao ML, Rédei J, and Andres AH (1993). Concurrence of cortex and platelet serotonin-2-receptor binding characteristics in the individual and the putative regulation by serotonin. *J Neural Transm (Gen Sect)* 93:27-35.

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