Immunomodulatory Effects of Antipsychotic Drugs in Whole Blood Cell Cultures from Healthy Subjects

Eun-Jeong Kim¹ and Yong-Ku Kim¹,*

¹Department of Psychiatry, Korea University Ansan Hospital, Korea University College of Medicine, Seoul, South Korea, Korea

Abstract: Background: We aimed to evaluate the effects of various antipsychotics on the in vitro production of C-reactive protein (CRP) in whole blood cell cultures from healthy volunteers. The evaluation was performed using haloperidol, quetiapine, clozapine, amisulpride, and chlorpromazine.

Methods: Antipsychotic agents were added to the participants' whole blood samples, and the resulting CRP levels were measured. For each agent, three different concentrations were tested: therapeutic concentration, one-tenth the therapeutic concentration, and ten times the therapeutic concentration. The differences in CRP concentrations before and after drug administration were investigated.

Results: The Friedman test showed that haloperidol, amisulpride, and chlorpromazine significantly increased CRP levels in the blood culture samples; however, clozapine and quetiapine did not increase CRP levels. In the case of chlorpromazine, elevated CRP levels were noted at all concentrations tested.

Conclusion: Our study suggests that some antipsychotics elevate CRP levels in vitro. These results agree with previous studies showing that antipsychotics have immunomodulatory effects. Future research will clarify our findings and our understanding of antipsychotic drugs and their impact on immune regulation.

Keywords: Schizophrenia, antipsychotic agents, inflammation, C-reactive protein, blood cell cultures, immune regulation.

1. INTRODUCTION

To date, the main hypothesis for the pathophysiology of schizophrenia has involved neurotransmitters including serotonin, dopamine, norepinephrine [1]. Both typical and atypical antipsychotics affect these neurotransmitter systems and their receptors, resulting in a reduction in positive and negative symptoms but also causing side effects. However, some patients show minimal improvement on antipsychotics due to the limited efficacy of antipsychotic drugs. Therefore, therapists have had difficulty treating this disease with antipsychotic drugs alone [2]. This limited efficacy of antipsychotic drugs suggests another pathophysiological background for schizophrenia other than the neurotransmitter system [3].

The neuroimmunologic hypothesis is one of the hypotheses that explain the limitations of the neurotransmitter hypothesis. The neuroimmunologic hypothesis suggests that inflammatory responses and neuroinflammatory interactions influence the pathophysiology of schizophrenia [3]. This hypothesis has been studied in relation to major depression [4], bipolar disorder [5], and other mental illnesses, with recent studies based on cytokines including interleukin-6 (IL-6) and interleukin-1 beta (IL-1β). IL-6 and IL-1β have increased serum concentrations in acute psychotic states [6, 7], and inflammatory markers are associated with the severity of positive symptoms in acute psychotic patients [8, 9]. Changes in inflammatory markers affect patients in many different ways. One of the most widely used inflammation markers, C-reactive protein (CRP), increases the activity of microglial cells in the central nervous system. Increases in CRP and activation of microglia activate the secretion of IL-6 and TGF-β (transforming growth factor-β). This process converts the tryptophan in astrocytes to pyruvic acid. After conversion, N-methyl-D-aspartic acid (NMDA) receptors are antagonized, and dopaminergic function is reduced in the limbic system through a decrease in brain-derived neurotrophic factor (BDNF). Dopaminergic hyperactivity in the limbic system is one of the factors that may explain schizophrenia and its positive symptoms [8].

Such dysfunction of immune responses, however, do not differ from the neurotransmitter hypothesis of schizophrenia.
As mentioned, tryptophan/kynurenine metabolism is related to NMDA pathway, kynurenic acid as an NMDA antagonist. Kynurenic acid can act in a critical way by reducing glutamatergic neurotransmission and thus affecting neurotransmitter mechanism of schizophrenia. Therefore, understanding neuroinflammatory pathways would help understand both neuroinflammatory and neurotransmitting background of schizophrenia [10].

Although increased inflammatory markers are consistently seen in schizophrenia, the effects of individual antipsychotic drugs on inflammatory markers are not consistent among studies. CRP elevation was found in a single trial of 25 acute psychotic patients treated with clozapine for 8 weeks compared with the control group [11]. CRP levels were higher in haloperidol-treated patients compared to the control group 3 months after drug administration, but CRP were higher in haloperidol-treated patients compared to the control group 3 months after drug administration, but CRP was not significantly increased at the 12-month follow-up compared to the control group [12]. In another study on aripiprazole, 15 subjects were included. In that study, CRP levels were slightly reduced but not to a statistically significant degree (p-value = 0.087) [13]. A study of 78 patients in their first episode with schizophrenia and treated with multiple antipsychotic drugs, a 4-week follow-up showed decreased WBC, decreased erythrocyte sedimentation rate (ESR), and decreased CRP [14]. Taken together, this shows that the results of drug administration varied from study to study, including whether elevation or reduction of inflammatory markers occurred.

The increase in inflammatory markers after the administration of antipsychotics can be explained in a variety of ways. First, antipsychotics can elevate lipid levels by blocking the histamine 1 receptor [12], which may be a factor in the early stages of the treatment (2-3 weeks of follow up). Second, weight gain after long-term administration of antipsychotic drugs may increase inflammatory markers. Weight gain can lead to metabolic changes, such as lipid profile changes and glucose metabolism changes, which can lead to a rise in the inflammation markers. Compared with short-term follow-up patients, long-term follow-up patients showed more weight gain and larger lipid and glucose profile changes, which supports this hypothesis [15].

However, this hypothesis accounts for the relationship between antipsychotic drugs and inflammatory markers in an indirect manner rather than through direct mechanisms such as antipsychotic agents in the peripheral or central nervous system. There is evidence that antipsychotics directly affect CRP and other inflammatory markers, one of which is the antipsychotic effect of prolactin and its tolerability [12]. When treated with olanzapine, patients had the highest prolactin levels during 3-month follow-up, which decreased at the 6- and 12-month visits. After 12 months, prolactin levels were similar to or lower than those seen in the first month [16]. Similar to this trend, CRP increases dramatically at a 3-month follow-up with no significant increase afterward [12].

In vitro studies, however, cannot isolate the effects of numerous variables including weight change, lipid profile, glucose profile, and other systemic effects of antipsychotics. Therefore, we designed this study to investigate the direct effect of antipsychotic drugs on inflammatory markers in samples from healthy patients. In vitro study would help us to understand the effect of antipsychotics on inflammatory reaction, which is a basis for understanding both neuroinflammatory and neurotransmitting basis of schizophrenia. Further understanding can help us understand the pathophysiology and treat the disorder.

Antipsychotic drugs, including haloperidol, quetiapine, clozapine, chlorpromazine, and amisulpride, were added at three concentrations (therapeutic level, one-tenth of the therapeutic level, and ten times the therapeutic level), and the resulting CRP levels were examined.

2. MATERIALS AND METHODS

2.1. Subjects

Eighteen healthy adult participants (9 males and 9 females) with a mean age of 31.94 ± 5.620 years (range, 22-39 years) were enrolled. None of the subjects had a personal or familial history of psychiatric or medical disease. No patients took any medications including antipsychotics, antidepressants, benzodiazepines, and other psychotropic treatments. A skilled psychiatrist interviewed each participant in a structured clinical interview using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (SCID). All patients scored less than 10 on the Beck Depression Inventory (BDI) and less than 40 on the State-Trait Anxiety Inventory (STAI). Participants underwent blood tests, including complete blood cell counts (CBCs), liver function tests, and kidney function tests, all of which were in the normal range. This protocol was approved by the Institutional Review Board (IRB) of Korea University Ansan Hospital. All participants gave written consent after a detailed explanation of the study.

2.2. Experimental

Blood sampling, complete blood count (CBC), and dopyring with antipsychotics.

Prior to blood sampling, participants abstained from smoking, drinking alcohol, and drinking caffeine for at least 12 hours before the blood sampling and fasted for 12 hours before the sampling. Whole blood sampling was done with the first sampling of the day from 8:00 am to 9:00 am and stored in heparinized tubes. To prepare for testing, 100 µL of healthy whole blood was added to 890 µL of Roswell Park Memorial Institute-1640 medium (Cambrex, East Rutherford, NJ, USA) supplemented with 10% fetal bovine serum (Welgene Inc, Daegu, Republic of Korea), 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA), 4 µg/mL phytohemagglutinin (PHA, Sigma-Aldrich, St. Louis, MO, USA), and 20 µg/mL lipopolysaccharide (LPS, Sigma-Aldrich, St. Louis, MO, USA) in a 24-well plate.

Each drug was prepared at three concentrations in 0.9% NaCl solution, including the therapeutic level, one-tenth the therapeutic level, and ten times the therapeutic level, with the therapeutic level as follows: haloperidol 1 ng/mL [17], quetiapine 5 ng/mL [18], clozapine, 40 µg/L [19], amisulpride 60 ng/mL [20], and chlorpromazine 20 ng/mL [21]. For each medication, 10 µL of the drug was added, and the culture was mixed using pipetting to avoid contamination. The sul-
tion was incubated in the air with 5% CO₂ for 48 hours then

<table>
<thead>
<tr>
<th>Medication</th>
<th>Control</th>
<th>Therapeutic Level × 10⁻¹</th>
<th>Therapeutic Level</th>
<th>Therapeutic Level × 10</th>
<th>Statistics*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haloperidol</td>
<td>-</td>
<td>0.1 ng/mL</td>
<td>1 ng/mL</td>
<td>10 ng/mL</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>475.06 ± 277.70</td>
<td>484.88 ± 279.34</td>
<td>477.29 ± 226.88</td>
<td>537.99 ± 231.56 †</td>
<td>χ² = 8.4, df = 3, P = 0.038</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>-</td>
<td>0.5 ng/mL</td>
<td>5 ng/mL</td>
<td>50 ng/mL</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>475.06 ± 277.70</td>
<td>560.64 ± 239.19</td>
<td>631.52 ± 265.33</td>
<td>640.87 ± 263.21</td>
<td>χ² = 6.53, df = 3, P = 0.089</td>
</tr>
<tr>
<td>Clozapine</td>
<td>-</td>
<td>4 μg/L</td>
<td>40 μg/L</td>
<td>400 μg/L</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>475.06 ± 277.70</td>
<td>615.56 ± 227.80</td>
<td>455.59 ± 270.63</td>
<td>449.53 ± 262.41</td>
<td>χ² = 6.38, df = 3, P = 0.095</td>
</tr>
<tr>
<td>Amisulpride</td>
<td>-</td>
<td>6 ng/mL</td>
<td>60 ng/mL</td>
<td>600 ng/mL</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>475.06 ± 277.70</td>
<td>506.47 ± 272.28</td>
<td>480.87 ± 119.37</td>
<td>585.37 ± 233.95 ‡</td>
<td>χ² = 13.35, df = 3, P = 0.004</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>-</td>
<td>2 ng/mL</td>
<td>20 ng/mL</td>
<td>200 ng/mL</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>475.06 ± 277.70</td>
<td>628.82 ± 251.21 §</td>
<td>664.60 ± 259.22</td>
<td>654.60 ± 250.79 §</td>
<td>χ² = 8.850, df = 3, P = 0.031</td>
</tr>
</tbody>
</table>

The results are presented as mean ± standard deviation of CRP concentration (in nanograms per milliliter). *Comparison of CRP concentration between control and treatment groups at three different drug concentrations using the Friedman test. †Wilcoxon test showed significantly increased CRP level when comparing the control and haloperidol dosing groups (therapeutic concentration × 10 group, Z = -1.970, p = 0.0488). ‡When the control and amisulpride dosing groups were compared, the mean concentration of the control group was 10⁻¹ and the reference concentration was the reference concentration × 10. Wilcoxon test showed that control group CRP concentration was (From low concentrations for each dose concentration Z = -2.482, p = 0.013, Z = -2.585, p = 0.010, Z = -2.534, p = 0.011).

2.3. Measurement of CRP

CRP levels were measured using the Human C-reactive Protein/CRP immunoassay kit (R & D systems, Minneapolis, USA) according to the manufacturer’s protocol. Briefly, 100 μL each of the sample and dilution reagent were mixed and incubated for 2 hours at 37°C. Next, 100 μL of detection antibody was added, followed by further incubation at room temperature for 2 hours. Then, 100 μL of substrate solution was added and incubated at 37°C for 20 minutes. For the final step, 50 μL of stop solution was added. The optical density of each sample was measured at 450 nm using a microplate reader (μQuant, Winooski, Vt). The intra-assay and interassay coefficients of variation were below 10% (Table 1).

2.4. Statistical Analysis

The mean and standard deviation of the CRP level of each sample was calculated. We estimated the difference between each sex. Differences in CRP levels before and after drug administration were investigated using the Friedman test. The Wilcoxon signed-rank test was used. The analyses were performed using SPSS Statistics software, version 24.0 (SPSS Inc, Chicago, Ill). The results were considered statistically significant at a P-value < 0.05.

3. RESULTS

The participants included 9 males and 9 females with an average age of 31.94 ± 5.62 years. There was no difference in the mean CRP level between men and women. (p = 0.231). For each antipsychotic drug, we used various concentrations of the drug and measured the resulting CRP levels. According to the Friedman test, haloperidol, amisulpride, and chlorpromazine increased CRP levels compared to the control group (haloperidol: χ² = 8.4, df = 3, P = 0.038, amisulpride: χ² = 13.35, df = 3, P = 0.004, chlorpromazine: χ² = 8.850, df = 3, P = 0.031). For quetiapine and clozapine at therapeutic concentrations, the Friedman test did not reveal any CRP differences compared to the control group (quetiapine: χ² = 6.53, df = 3, P = 0.089, clozapine: χ² = 6.38, df = 3, P = 0.095). The Wilcoxon test showed elevated levels of CRP in response to haloperidol and amisulpride at ten times their therapeutic concentration (haloperidol: Z = -1.970, p = 0.0488, amisulpride: Z = -2.275, p = 0.023), although there were no significant changes at the therapeutic concentration (haloperidol: χ² = 0.37, df = 3, p = 0.711, amisulpride: χ² = -0.352, df = 3, p = 0.521) or at the therapeutic concentration × 10⁻¹ (haloperidol: Z = -0.37, p = 0.711, amisulpride: Z = -1.913, p = 0.056). For chlorpromazine, there were no significant differences at any of the studied concentrations (therapeutic concentration × 10⁻¹: Z = -2.482, p = 0.013, therapeutic concentration × 10: Z = -2.585, p = 0.010, therapeutic concentration ×10: Z = -2.534, p = 0.011).

4. DISCUSSION

Our study examined in vitro CRP levels in healthy subjects before and after the administration of antipsychotic drugs. The different antipsychotic drugs had varying effects, namely that haloperidol, amisulpride, and chlorpromazine significantly increased CRP levels but quetiapine and clozapine did not. Haloperidol and amisulpride elevated CRP levels when given ten times their treatment concentrations, and chlorpromazine elevated CRP levels at all three concentrations studied.
Changes in inflammatory markers after administration of various antipsychotics have been studied. For clozapine, CRP levels were increased at a 3-month follow-up, but there was no statistically significant change at 12-month follow-up [12]. In the case of ziprasidone, an 18-month follow-up showed an increase in CRP [22]. Additionally, olanzapine and quetiapine elicit higher CRP elevations compared with perphenazine, risperidone and ziprasidone [23]. Differences between antipsychotics and many other inflammatory markers, including cytokines, have also been studied [24]. Because elevation of CRP and other inflammatory markers correlates with elevation in markers associated with metabolic syndrome, it is assumed that inflammatory marker elevation is related to changes associated with metabolic syndrome. However, that study divided subjects into two groups by initial CRP level [24], so further study is required.

Our *in vitro* study supports the suggested hypothesis that antipsychotics are associated with inflammatory reactions. A recent Indian study showed RP and IL-6 elevation in schizophrenia patients taking antipsychotic medications, which is inconsistent with our study results [25]. Previous studies have suggested that antipsychotics may affect cell damage and inflammatory reaction [11], but the specific mechanism is not yet known.

One of the most famous atypical antipsychotic drugs, clozapine, can increase the number of white blood cells upon administration. This effect is related to many patient factors including being male, smoking, and co-administered drugs such as lithium [26]. Clozapine increases leukocyte counts for months or years after administration. This may be due to the correlation between granulocyte colony stimulating factor (G-CSF) and TNF-α elevation, as elevated TNF-α may also affect CRP elevation in the liver [27]. Thus, after administration of antipsychotics, elevations in TNF-α and WBC may lead to increased CRP levels.

Changes in cytokines also support the purported immunomodulatory effects of antipsychotics. Various studies have reported the effects of antipsychotics on the alteration of inflammatory markers, including cytokines, in schizophrenic patients [28]. In many *in vivo* studies, elevations or decreases in cytokines were observed. One *in vivo* study showed that IL-6, IL-10, IFN-γ, and IL-1 receptor antagonists (IL-1RA) increase after the use of clozapine. Elevations in IL-1RA were also found after the use of haloperidol [29]. In another *in vivo* study, long-term clozapine administration induced IL-2R elevation. IL-1RA, an endogenous anti-inflammatory agent, was also increased in the studies [30, 31]. However, few specific patient features, such as catatonia and aggressiveness, showed a relationship with CRP levels [28]. Recent *in vitro* studies using ziprasidone, based on previous theories that ziprasidone can cause hypersensitivity reactions, showing direct activation of macrophages [22] along with increased IL-1, IL-6, TNF-α, and IFN-γ. Ziprasidone also affects the expression of cytokine genes, demonstrating its inflammatory effects in the periphery.

One *in vitro* study in 2015 provided similar result of our study. Atypical antipsychotics, haloperidol and risperidone activated *in vitro* inflammatory response *in vitro* setting [32]. The classical inflammatory response via macrophage was found in the environment, independent of other physiological responses of *in vivo* setting. The authors suggest the effect of few antipsychotics on macrophage activation enables inflammatory reaction. In the study, medication triggered an increase in inflammatory cytokines as the medication concentration raises. The study used nitrous oxide level to see the macrophage activity and measured cytokine IL-1β, IL-6, TNF-α to see the inflammatory responses. These results can also support our study results with a biological background.

The study also applies the explanation *in vivo* setting, suggesting that antipsychotics may exacerbate the peripheral tissue inflammatory process, resulting in obesity and other endocrine or metabolic disturbances. Another review also insisted that both pro- and anti-inflammatory effect of antipsychotics may be due to various interactions with biological systems or individual lifestyle factors *in vivo* setting [33].

Our findings show that CRP is increased after the administration of antipsychotics and supports the theory of the immunomodulatory effects of antipsychotics. Antipsychotics affect the levels of WBC and CRP as well as IL-6, IL-10, and IFN-γ through the action of TNF-α. Some antipsychotics also elevate proinflammatory cytokines through macrophage proliferation, which also explains the rise in inflammation markers. However, a decline in CRP has been found in several animal studies. The administration of risperidone to rats causes a significant long-term decrease in CRP [34]. Decreased kynurenic acid levels after administration of antipsychotic medications enable the anti-inflammatory effects of antipsychotic drugs [35]. Given these results, there is some uncertainty about the association between antipsychotics and CRP or inflammatory markers. This discrepancy supports the immunomodulatory effect of antipsychotics, and further studies are needed to assess these correlations.

The reason why differences among antipsychotic medications exist is a bit obscure. According to the previous studies, each antipsychotic showed different immunologic reactions. Chlorpromazine, for example, is known to have high proautoimmune potential. Previous studies found the chlorpromazine to have pro-autoimmune potential and thus cause autoimmune lesions through humoral immune pathway. In constant with this aspect, few cases related to drug-related lupus, autoimmune hematologic diseases were reported [36]. This pro-autoimmune potential of specific medication can partly explain the elevation of pro-inflammatory cytokines and relation with inflammatory actions, in few antipsychotics, not in all antipsychotics.

When thinking of tested concentration, the response in higher level can reflect dose-responsive relation. From our result, amisulpride and haloperidol showed CRP elevation in higher concentration, rather than lower concentrations. We can assume that pro-inflammatory reaction may occur more frequently in higher concentration in two antipsychotics. Chlorpromazine, however, showed CRP elevation in all studied concentrations. It may be due to the differences in immunomodulatory properties among antipsychotics, but the underlying mechanism of this difference is unclear. Further studies are required to clarify this difference.

The limitations of our study are as follows. First, it is difficult to compare clinical CRP levels with *in vitro* CRP
levels after drug administration, especially considering the varied effects of different antipsychotics. Second, this study was conducted using healthy subjects, not acutely psychotic patients or schizophrenic patients, so it does not account for physiological changes in schizophrenic or mentally ill patients. Third, CRP levels reflect the immunological status of the periphery but may differ from that of the central nervous system. Lastly, a small number of sample size is another limitation of our study. A study with larger sample would be required to support our result.

Regardless of the limitations mentioned above, our study is meaningful because it directly measures the effects of antipsychotics on inflammatory markers that were replicated in this study. A recent study from India showed an elevation of both CRP and IL-6 in medicating schizophrenia patients, which is in constant with our result. An Indian study found elevated CRP of schizophrenic patient, both before and after medicating, compared to the control group. Both in vivo and in vitro setting showed elevation of CRP. A previous study with clozapine also showed an elevation of hsCRP after medication administration [11]. It was thought to be a transient acute-phase response immediately after medication administration. The definite mechanism of this elevation is yet unclear and needs further studies.

Our study was still meaningful for isolating the immunomodulatory effects of antipsychotics and exclude their systemic effects. We predict that our results will deepen the understanding of antipsychotic drugs, their relationship with immune responses, the pathophysiology of schizophrenia, and ultimately, the mechanism of treatment.

CONCLUSION
We examined CRP levels in healthy subjects after the administration of antipsychotics and compared them according to the drug concentrations. Haloperidol, amisulpride, and chlorpromazine administration elicited a significant increase in CRP levels. For haloperidol and amisulpride, the effect was only seen at 10 times the therapeutic concentration, but chlorpromazine increased CRP at all of the concentrations studied. These results show the direct effects of antipsychotic drugs on inflammatory markers by excluding other systemic effects. This study supports the possibility of an immunomodulatory effect of antipsychotic drugs, including increasing pro-inflammatory cytokines, as shown in previous studies.

CURRENT & FUTURE DEVELOPMENTS
Our study results suggest a possible immunomodulatory effect of antipsychotic drugs. Based on the results of this study, it is necessary to continue to study the detailed process of inflammatory reactions related to antipsychotic drugs, which will broaden the understanding of the pathophysiology of schizophrenia.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE
This study was reviewed and approved by the Ethics Committee of the Korea University Medical Center, (IRB: 2007AAS0034), Seoul South Korea.

HUMAN AND ANIMAL RIGHTS
No animals were used in this research. All human procedures were followed in accordance with the ethical standards of the Institutional Ethics Committee (Human Studies) and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION
The subjects signed the written informed consent form.

AVAILABILITY OF DATA AND MATERIALS
Not applicable.

FUNDING
None.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS
Eun-Jeong Kim wrote the first draft of the article and Yong-Ku Kim revised the article.

REFERENCES


[25] Gurung J, Chamlangi D, Bera NK, Chaudhuri TK. Elevated levels of C-reactive protein and IL-6 among the antipsychotic medicating schizophrenia patients of Siliguri, West Bengal, India. Nord J Psychiatry 2018; 72(4): 311-17. [PMID: 29464976]


