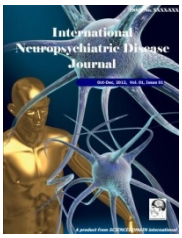

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Effect of Different Antipsychotics on Cytokine Production After Immunologically Stimulated PBMC Culture

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Author's contribution

This work was carried out solely. Author MMAA designed the study and wrote the protocol, collected data and performed the statistical analysis and wrote the first draft of the manuscript. Author MMAA managed the literature searches, analyses of the study and finalize the manuscript. Author read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To investigate the effect of different antipsychotics on cytokine production in immunologically challenged Peripheral Blood Mononuclear Cell (PBMC) culture.
Study Design: In vitro cell culture study to determine cytokine (IL-4, IL-10 and IFN-γ) level.
Place and Duration of Study: Department of Pharmacy, North South University, Dhaka between January 2013 and April 2013.
Methodology: Blood sample was collected from 22 healthy volunteers. Peripheral Blood Mononuclear Cells were separated and culture was prepared. The culture was stimulated with either LPS (lipopolysaccharide) or poly(I:C) (polyinosinic:polycytidylic acid). Stimulated PBMC culture was treated with typical antipsychotic (Haloperidol) and atypical antipsychotics (Clozapine, Quetiapine, Risperidone). Pro-inflammatory (IFN-γ) and anti-inflammatory (IL-4, IL-10) cytokine levels were determined from the stimulated PBMC culture and stimulated plus antipsychotic treated PBMC culture.
Results: Typical antipsychotic; Haloperidol and atypical antipsychotics; Clozapine, Quetiapine, Risperidone significantly (P = .05) enhance IL-10 production but not IL-4 in the LPS and poly(I:C) stimulated PBMC culture. IL-10 production was robust in LPS stimulated PBMC culture than the poly(I:C) stimulated culture. Typical and atypical both antipsychotics significantly (P = .05) reduce increased IFN-γ level in the LPS and poly(I:C)

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stimulated PBMC culture.
Conclusion: Typical and atypical antipsychotics were successfully alters immune function by the suppression of pro-inflammatory cytokine (IFN-γ) levels and elevation of anti-inflammatory cytokine (IL-10).

Keywords: Schizophrenia; cytokine; antipsychotic; lipopolysaccharide.

1. INTRODUCTION

A plenty of research studies try to prove the involvement of immune system in schizophrenia. Methods to approach this question are varied manifold with some of them describe a) polymorphisms in genes associated with immune function, b) prenatal infection, c) disrupted cytokine networks in adulthood and d) changes in circulating peripheral immune cells.

In case of inflammation, increased levels of immune cells travel through the blood stream and migrate into the peripheral tissues. Circulating blood cells including monocytes and T-lymphocytes are indicators for the presence of inflammatory processes in schizophrenia. Evidence suggests an increased number of monocytes in schizophrenia [1,2]. Studies about leukocytes display inconsistent data. No difference [1,3,4], increased [5-7] and decreased [8-10] number of T-cells were reported previously. A similar frequency of T-cell subsets between inflammatory and non-inflammatory process in schizophrenia supports the hypothesis of low level inflammatory response in the pathogenesis of the illness [11]. Mice deprived of mature T-cells manifests cognitive and behavioural abnormalities, which are remediable by T-cell restoration [12]. This indicates how a properly functioning immune system and a normal T-cell level could help to restore psychotic symptoms. Despite the inconsistent result of T-cells, there are a number of studies have been conducted concerning B-cells. Hyperfunction [13] and elevated B-cells [1,14] were reported previously. A recent study yielded a shift towards B cell immunity [10]. Even though the results are inconsistent, the huge amount of alterations in immune cells clearly indicates some sort of inflammation in schizophrenic patient (SCP) compared to the control.

1.1 Cytokine Networks in Schizophrenic Patients

Peripheral changes in cytokines levels of SCP have been vigorously reported. Alterations of cytokine level, cytokine receptors and cytokine activity modifiers have been observed in the blood and cerebrospinal fluid (CSF) of SCP. However, the data is often very inconsistent. Some articles report increased levels of IL-6, TNF-α [3,15-18] and decreased levels of IL-2 [19,20] others report no change in these cytokine levels [21,22]. In 2008, a systematic quantitative review demonstrated only significant effect sizes for IL-6 and IL-2 levels. The meta-analysis yielded an increase in-vivo peripheral level of IL-6 and a decrease in-vitro IL-2 but no significant effect size for TNF-α [23].

There were no consistent evidence for pro-inflammatory cytokine; IFN-γ and anti-inflammatory cytokines; IL-4 and IL-10. Kaminski et al. in 2000 have reported a decreased IL-4 level after in-vitro stimulation [22]. A lower detection rate of plasma IL-4 [18,24,25] and no difference of IL-4 compared to control persons [26]. An increased level of IFN-γ in the serum and plasma of SCP [22,24,26] was found. Other studies stated a decreased IFN-γ level in whole blood cell cultures [4,27]. Inglot et al. in 1994 stated that there may have a

connection between IFN-γ and psychopathology in schizophrenia [28]. Patients with positive symptoms had elevated production of IFN-γ while negative symptoms were associated with decreased IFN-γ production [28]. This is inconsistent with the assumption that a decrease in IL-2 and IFN-γ could be only seen in paranoid schizophrenics [4]. An increase of IL-10 levels in non-paranoid schizophrenic patients compared to healthy controls has also been reported [29] whereas other study did not support this finding [30]. A strong relation between CSF levels of IL-10 and negative symptoms in SCP have been observed. This study suggests that the severity of negative symptoms is positively correlated with IL-10 concentrations in CSF. A shift from Th1 immunity to Th2 immunity was proposed as pathophysiological mechanisms in schizophrenia [31,32]. This shift would be indicated by a lower IFN-γ/IL- 4 ratios. Some researcher supports this hypothesis by finding a lower IFN-γ/IL-4 ratio [26] others however found a higher ratio. This finding might indicate that the underlying pathology is associated with the disturbances in the balance between pro and anti-inflammatory cytokines and a shift in Th1 and Th2 cells. Alternatively, the shift could also arise from the antipsychotic treatment [25]. Recently, it has been demonstrated that typical and atypical antipsychotic drugs have effects on the production of cytokines [15,33].

1.2 Aim of the Study

Evidence suggests that the association of schizophrenia with the immune system deregulation. Now, it is needed to get a clear picture about the underlying mechanisms of the inflammatory responses and processes after the administration of antipsychotic drug in SCP. Therefore, the aim of our current study is to examine the immunomodulatory effects of typical (Haloperidol) and atypical (Clozapine, Risperidone, Quetiapine) antipsychotic agents. We were aimed to investigate the effects of those agents on the unstimulated and stimulated production of pro-inflammatory cytokine IFN-γ and anti-inflammatory cytokines; IL-4, IL-10.

2. MATERIALS AND METHODS

2.1 Subjects

Blood samples were collected from twenty two healthy volunteers (11 women; age range: 19-61 years; mean age = 33.82 ± 2.63 years) for the assay of cytokine production. All subjects gave a written consent and the experimental procedure was previously approved by the ethics committee of the Department of Pharmacy, North South University, Bangladesh. Subjects were excluded on the basis of following criteria. a) subjects with a past or present history of psychiatric disorders; b) subjects who ever had been taking major psychotropic medications, e.g. antidepressants and antipsychotics; c) subjects with drug (alcohol and any other drug of dependence) abuse; c) subjects with any medical, e.g. endocrine, immune, metabolic disorders, such as diabetes, autoimmune disorders, inflammatory bowel disease, acquired immunodeficiency syndrome; d) subjects who currently (2 weeks prior to the first blood sample) suffered from an infectious, allergic or inflammatory response. The subjects were abstained from caffeine, alcohol and nicotine for at least 8 hour before blood samplings.

2.2 Methods

Cytokines can be measured under various in vitro and in vivo conditions in the body fluids of schizophrenic patients. They include serum, whole blood, plasma, cerebrospinal fluid (CSF)

and in vitro methods like peripheral mononuclear blood cells cultures (PBMC). PBMCs are purified lymphocytes, consisting mostly of leukocytes and monocytes [34].

2.2.1 Blood collection

Venous blood (18 ml) was collected into heparinized tubes at average 8:00 AM in the morning. Subjects were fasted for overnight. The effects of antipsychotic agents on the production of cytokine were investigated by stimulating peripheral blood mononuclear cells (PBMC).

2.2.2 Peripheral blood mononuclear cell separation

Blood obtained from normal donors was diluted 1:1 with sterile phosphate-buffered saline (PBS), layered over Ficoll-Hypaque and centrifuged at 1500 rpm for 30 minutes at room temperature. The interphase layer of PBMCs was drawn out. Isolated PBMC were incubated in RPMI medium-1640 (Sigma R-8005) with l-glutamine and Phenol Red containing 1% penicillin (Sigma) at micro-titration desks in concentrations 10^6 cells per well. Samples were incubated for 72 h in a humidified atmosphere at 37ºC, 5% CO_2 to get peak cumulative responses for most cytokines. The plates were centrifuged at 1500 rpm for 8 minutes after incubation. Supernatants were taken of carefully under sterile conditions, divided into Eppendorf tubes and frozen immediately at -70ºC until they were thawed for assay.

2.2.3 Immune challenge and the addition of antipsychotic medication

Lipopolysaccharide (LPS) and polyinosinic:polycytidylic acid (polyI:C) at a concentration (1 mg/ml) was added with PBMC culture for bacterial and viral stimulation respectively. In untreated antipsychotic condition, 75 µl PBMC culture was added with 225 µl stimulant medium (LPS or polyI:C) to make a final volume of 300 µl and placed into 24 well sterile plates. In antipsychotic treatment condition, 10 µl antipsychotic drug (Haloperidol, Clozapine, Quetiapine and Risperidone) solution and 75 µl PBMC culture was added with 215 µl stimulant (either LPS or polyI:C) medium to make final volume 300 µl. The concentration of all antipsychotic drugs was 1 mg/ml. Haloperidol, Clozapine, Quetiapine and Risperidone were collected from a local pharmaceuticals company in Bangladesh. The amount of cytokine (IL-4, IL-10, and IFN-γ) in PBMC culture supernatants were quantified by ELISA method. In our study, intra-assay CV values were less than 8% and the limits of detection (LOD) were: IL-10: 10 pg/ml; IFN-γ: 1.03 pg/ml and IL-4: 0.39 pg/ml.

2.3 Statistics

In order to investigate the effects of antipsychotic drug on the cytokine (IL-4, IL-10 and IFN-γ) level in immune stimulated PBMC culture repeated measure ANOVA was conducted. Repeated measure ANOVA examine (1) within-subject variability with effects of antipsychotic drugs and/or effects of LPS/poly(I:C) treatment as temporal condition; and (2) between-subject variability with gender as a factor. The difference was considered significant, when p value was less than or equal to 0.05. Data were represented as means ± SEM (Standard Error Mean). SPSS (version 16.0) was used for statistical analysis.

3. RESULTS AND DISCUSSION

We measured the cytokine (IL-10, IL-4 and IFN-γ) production after PBMC culture stimulation and antipsychotic treatment.

3.1 Effect of Antipsychotic Drug on IL-10 Production in Immune Stimulated PBMC Culture

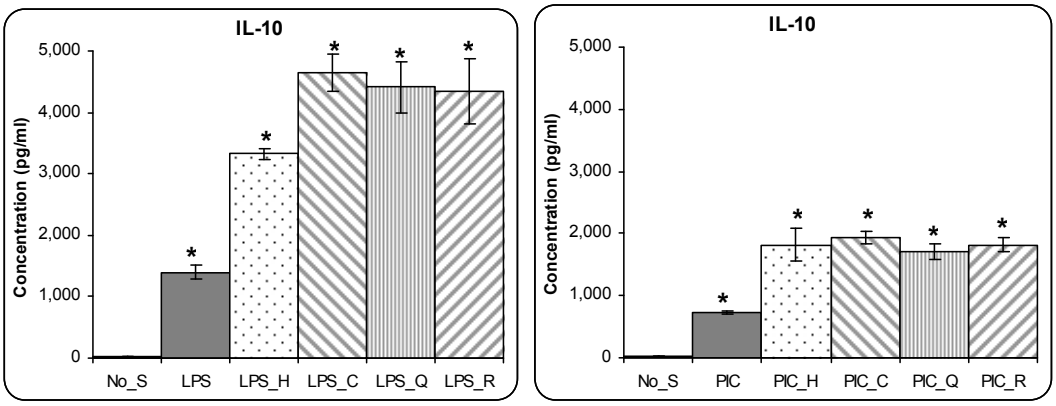

Fig. 1. Effect of different antipsychotic on IL-10 production after LPS (Left) and poly(I:C) (Right) stimulated PBMC culture (No_S: No Stimulation, LPS: Lipopolysaccaride, PIC: polyinosinic:polycytidylic acid, H: Haloperidol, C: Clozapine, Q: Quetiapine, R:Risperidone)

** P < 0.05; Data is represented as Mean ± SEM (Standard error of means).*

Typical antipsychotic Haloperidol significantly increase IL-10 production in PBMC culture when stimulated by LPS ($F[1, 19] = 62.87$, $P = .05$) (Fig. 1) or poly(I:C) ($F[1, 19] = 15.51$, $P = .05$). Atypical antipsychotic; Clozapine ($F[1, 19] = 71.08$, $P = .05$) Quetiapine ($F[1, 19] = 24.43$, $P = .05$) and Risperidone ($F[1, 19] = 37.25$, $P = .05$) significantly increase IL-10 production in PBMC culture when stimulated by LPS. Clozapine ($F[1, 19] = 59.99$, $P = .05$), Quetiapine ($F[1, 19] = 23.51$, $P = .05$) and Risperidone ($F[1, 19] = 62.75$, $P = .05$) significantly increase IL-10 production in PBMC culture when stimulated by poly(I:C).

3.2 Effect of Antipsychotic Drug on IFN-γ Production in Immune Stimulated PBMC Culture

Haloperidol significantly decrease IFN-γ production in PBMC culture when stimulated by LPS ($F[1, 19] = 37.66$, $P = .05$) or poly(I:C) ($F[1, 19] = 29.20$, $P = .05$) (Fig. 2). Clozapine ($F[1, 19] = 36.55$, $P = .05$), Quetiapine ($F[1, 19] = 40.48$, $P = .05$) and Risperidone ($F[1, 19] = 19.58$, $P = .05$) significantly decrease IFN-γ production in PBMC culture when stimulated by LPS. Clozapine ($F[1, 19] = 27.05$, $P = .05$), Quetiapine ($F[1, 19] = 26.74$, $P = .05$) and Risperidone ($F[1, 19] = 27.71$, $P = .05$) significantly decrease IFN-γ production in poly(I:C) stimulated PBMC culture.

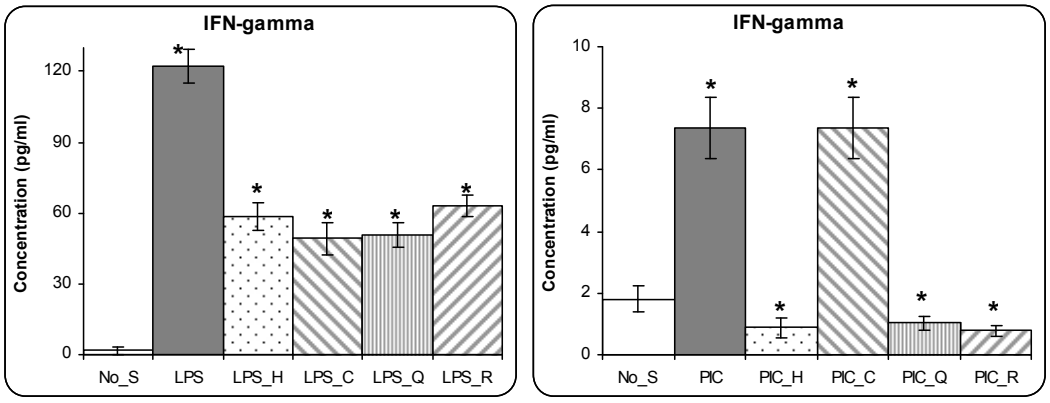

Fig. 2. Effect of different antipsychotic on IFN-γ production after LPS (Left) and poly(I:C) (Right) stimulated PBMC culture (No_S: No Stimulation, LPS: Lipopolysaccaride, PIC: polyinosinic:polycytidylic acid, H: Haloperidol, C: Clozapine, Q: Quetiapine, R:Risperidone)

** P < 0.05; Data is represented as Mean ± SEM (Standard error of means).*

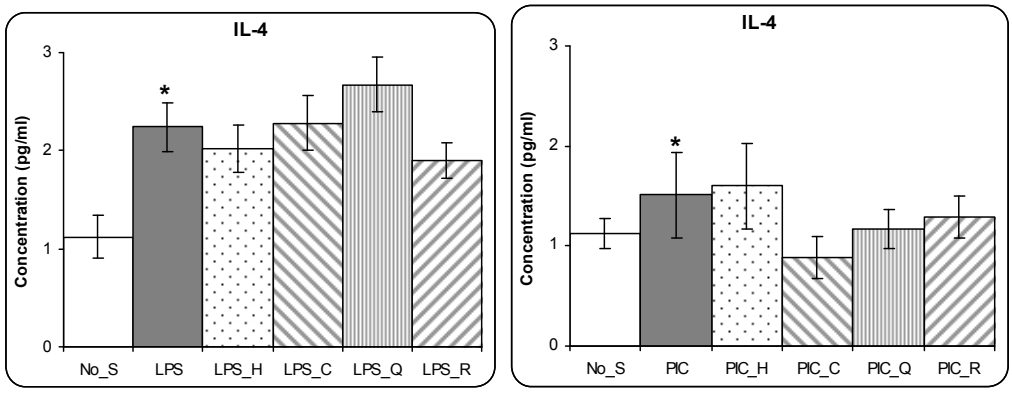

Fig. 3. Effect of different antipsychotic on IL-4 production after LPS (Left) and poly(I:C) (Right) stimulated PBMC culture (No_S: No Stimulation, LPS: Lipopolysaccaride, PIC: polyinosinic:polycytidylic acid, H: Haloperidol, C: Clozapine, Q: Quetiapine, R:Risperidone)

** P < 0.05; Data is represented as Mean ± SEM (Standard error of means).*

3.3 Effect of Antipsychotic Drug on IL-4 Production in Immune Stimulated PBMC Culture

Typical and atypical both antipsychotics have not shown any significant effects on IL-4 production in either LPS or poly(I:C) stimulated PBMC culture (Fig. 3).

The main findings of this study are; atypical antipsychotics (Clozapine, Quetiapine and Risperidone) enhances the production of IL-10 and lowers increased IFN-γ level in both LPS and poly(I:C) challenged PBMC culture separately. Both typical and atypical antipsychotic has no effect in the production of IL-4 in either LPS challenged or poly(I:C) challenged

PBMC culture. Both types of antipsychotic agents were successful to alter immune function. In one hand, antipsychotic drugs suppress pro-inflammatory cytokine (IFN-γ) level and on the other hand increase anti-inflammatory cytokine (IL-10). Thus antipsychotic agents might produce both type of immunomodulatory effects and may contribute beneficial effect in SCP.

The findings of the present study are consistent with the previous study. Haloperidol enhances anti-inflammatory cytokine (IL-10) level in PBMC culture [38]. This is in contrast to another study that found no upregulation of IL-10 in LPS stimulated and Haloperidol treated mice [46]. Contradictory results obtained from studies on IFN-γ either stimulating [41] or inhibitory [37,38] effects on in-vitro cell cultures. A recent study reported a weak effect on the inhibition of harmful nitric oxides (NO) by IFN-γ activated microglia [45]. Haloperidol seems to normalize increased IL-2 plasma /serum levels [36] and to inhibit mitogen-stimulated IL-2 production in PBMC culture [37,38]. The effect of Haloperidol was particularly evident in patients with a predominance of positive symptoms [39]. Haloperidol reduces TNF-α production on LPS stimulated monocytes [40], exert no effect on IL-6 concentration in Serum/Plasma [36,42,43] as well as CSF [44] in SCP.

Effect of atypical antipsychotic on inflammatory compounds in schizophrenia has also been reported. Robust increase of IL-10 level in serum [46] and IFN-γ suppression in PBMC culture [51] by Clozapine was observed. Maes et al (1994) showed that Clozapine modulates IL-2 level [33]. Quetiapine reduces IL-6, IL-17 level in collagen-induced arthritis in animal model [49]. It also decreases T-cell populations in lymph nodes and spleens in animal model [50]. There are no specific guidelines regarding the immunomodulatory effects of Risperidone. Some studies report decreased plasma levels of IL-2, IL-6 and IFN-γ [36, 47, 48], other studies report no differences [36, 48]. Recently a study revealed strong beneficial effects of Risperidone on IFN-γ stimulated microglia where Risperidone was shown to inhibit the production of NO and some other pro-inflammatory cytokines, including TNF-α and IL-6 [45]. Increased level of IL-10 [48] unchanged [48] and lower [52] level of IL-4 have also been found in the plasma of first episode SCP. A recent study report that IL-4 level is increased by both typical and atypical antipsychotics in whole blood [53].

In this study, only one concentration of all antipsychotic drugs was used. One may ask question regarding why one concentration was used. This concentration was chosen from previous study. Antipsychotic drug concentrations in the blood during the treatment of SCP were measured previously [35]. This concentration was chosen on the basis of their bioavailability after taking orally. Different concentration of antipsychotic drug can be employed in future trial to see the level of anti-inflammatory cytokine changes in PBMC culture.

One of the most important points of this study is to stress in healthy subjects rather than in the SCP. Blood sample from the healthy volunteer was suitable and produce significant cytokines after the stimulation with LPS and poly(I:C). This situation can be matched with the SCP where poor immune system is exists.

Another important question one may have that how LPS and poly(I:C) stimulates PBMC. It is well established that LPS has a specific receptor which is known as TLR-4 (Toll like receptor 4) receptor. Poly(I:C) has also a specific binding and recognizing site which is known as TLR-3 (Toll like receptor 3) receptor. A cascade of second messenger system activated after the binding of poly(I:C) with TLR-3 and LPS with TLR-4 receptor in the cell membrane. LPS and poly(I:C) both activates nuclear localization of transcription factor nuclear factor kB (NF-

kB) and subsequent activation of genes in the inflammatory pathways. Thus, inflammatory cytokines are produced from this second messenger system activation.

Finally, this study gives an idea for designing biomarker-based tests for molecular profiling at different stages of schizophrenia.

4. CONCLUSION

Immune system involvement in schizophrenia is a well established idea. Study regarding the immunosuppressive action of antipsychotic drugs provides contradictory and unsatisfactory proof. This study might be strengthening the idea regarding immuomodulating activity specifically immune system enhancing ability of antipsychotic drugs. Further studies in immunomodulation could also lead to the development of a novel target discovery strategy for schizophrenic patient. More importantly, targeting the inflammatory component of schizophrenia should be monitor through the progression of schizophrenic disease. Cytokine disturbance pathways and processes are needed to be explored.

CONSENT

Participants were informed about the purpose of the study. Their consent was taken before the collection of blood sample.

ETHICAL APPROVAL

Author hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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