Original Article

The Effect of Ibuprofen on Cytokine Production by Mononuclear Cells from Schizophrenic Patients

(ibuprofen / interleukins / mononuclear cells / schizophrenic patients)

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Abstract. The existence of a restrained inflammatory state in schizophrenic individuals posed the question whether anti-inflammatory drugs may exert antipsychotic effects. Therefore, the effect of ibuprofen (IB) on cytokine production by human peripheral blood mononuclear cells (PBMC) from schizophrenic patients was examined and compared to that of healthy subjects. PBMC from 25 schizophrenic patients and 24 healthy volunteers were incubated for 24 h with lipopolysaccharide (LPS) in the absence or presence of various concentrations of IB. The levels of IL-1β, IL-6, TNF-α, IL-10 and IL-1ra in the supernatants were tested applying ELISA kits. The secretion of TNF-α by cells from schizophrenic patients was significantly lower compared with controls. IB caused stimulation of TNF-α and IL-6 production by cells of the two groups and enhanced IL-1β secretion by cells from schizophrenic patients. IB inhibited IL-1ra and IL-10 generation by cells from the two groups. Without IB, IL-1ra secretion was negatively correlated with the disease severity, while 200 µg/ml of IB positively correlated with the PANSS total score. IL-10 production was positively correlated with the PANSS positive subscale score both in the absence or presence of IB. The findings suggest that the effect of IB on the production of inflammatory cytokines may benefit the health of schizophrenic patients.

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Abbreviations: CGI – Clinical Global Impressions scale, CM – complete medium, CNS – central nervous system, GAF – Global Assessment Functioning, IB – ibuprofen, LPS – lipopolysaccharide, PANSS – Positive and Negative Syndrome Scale, PBMC – peripheral blood mononuclear cells, PBS – phosphate-buffered saline, sIL-2R – soluble interleukin-2 receptor.

Introduction

The pathogenesis of schizophrenia may be linked, at least in part, to the immune system. Several hypotheses concerning immune-related disorders, such as infections and autoimmune inflammatory diseases, have been suggested as aetiological factors for the occurrence of schizophrenia (Sirota, 1990; Brown and Susser, 2002; Eaton et al., 2006; Potvin et al., 2008). In that sense, the activity of cytokines as crucial mediators of the immune system and central nervous system (CNS) and the cross-talk between them became an area of major interest. Cytokines can modify the metabolism of neurotransmitters to influence neural development, as well as acute and chronic neurodegeneration (Sirota et al., 1995, 2005; Rothwell and Hopkins, 1995; Maes et al., 1995; Dinarello, 1996). It has been found that lymphocytes from schizophrenic patients produce lower amounts of IL-2, whereas the concentration of this cytokine in the cerebrospinal fluid is increased (Bessler et al., 1995; Ganguli et al., 1995; Maes et al., 1995).

Since peripheral application of IL-2 enhanced neurotransmission of catecholamines in the rat frontal cortex and hippocampus, it was suggested that IL-2 may play an important role in the pathophysiology of schizophrenia (Zalcman et al., 1994). In view of the fact that elevated levels of the soluble interleukin-2 receptor (sIL-2R) have been found repeatedly in the serum of schizophrenic patients, its detection has become one of the standard examinations for recognition of immunological activity in schizophrenia (Gaughran et al., 2002; Sirota et al., 2005). In addition, it has been found that atypical antipsychotic agents increase serum sIL-2R, whereas typical antipsychotic drugs have no effects on this receptor (Haack et al., 1999; Schwarz et al., 2001). According to Rothermundt et al. (1998), acutely ill schizophrenic patients showed increased IL-2 and IFN-γ, while IL-10 and sIL-2R levels remained unchanged. The authors concluded that the deficient production of Th-1 cytokines in schizophrenia is neither due to changes in the number of immunocompetent cells, nor to coun-

ter-regulation of the Th-2 cytokine IL-10. These findings support the hypothesis that schizophrenia is linked to activation of cell-mediated immunity.

It is notable that in schizophrenic patients there is increased secretion of IL-1 in the CSF and by their PBMC (el-Mallakh et al., 1993; Sirota et al., 1995). As for other cytokines, increased serum IL-6, IL-10 and TNF-α levels have been reported to be associated with the course, treatment, or progression of the illness (Ganguli et al., 1994; Naudin et al., 1997; Lin et al., 1998; Maes et al., 2000). NSAIDs exert immunomodulatory effects by interfering with human monocyte and T-lymphocyte activation, proliferation and cytokine synthesis (Paccani et al., 2002; Härtel et al., 2004). Ibuprofen (IB), a 2-arylpropionic acid derivative, is a Cox-1/Cox-2 nonselective inhibitor of prostaglandin synthesis and possesses immunoregulatory activity. The studies of the effect of IB on cytokine production are controversial. While some investigators have found increased pro-inflammatory cytokine secretion induced by IB, others have reported that it was reduced or unchanged (Kay et al., 1989; Endres et al., 1996; Mansilla-Rosello et al., 1997; Qui et al., 1997; Stuhlmeier et al., 1999). Based on the fact that Cox inhibitors exert an immunomodulatory effect and that the immune system is closely involved in aetiopathology of schizophrenic patients, we examined the effect of ibuprofen on cytokine production by PBMC from schizophrenic patients and compared the results to those of healthy subjects.

Material and Methods

Patients

The study was approved by the Ethics Committee of the Abarbanel Mental Health Center. The participants gave their written informed consent. Twenty-five schizophrenic inpatients who met the DSM-IV (American Psychiatric Association, 2000) diagnostic criteria for schizophrenia, aged 24–61 years (mean age 37.96 ± 9.46), were enrolled in the study. Twenty-four healthy blood bank donors aged 25–52 years (mean 36 ± 9.7) served as controls. There was no significant difference in age and male-to-female ratio between the patients and controls. Illness duration ranged between 1–45 years (mean 14.32 ± 9.86). Patients with other Axis I diagnoses besides schizophrenia were excluded from the study. The schizophrenic patients showed normal physical examination, blood values (complete blood count, liver enzymes, serum electrolytes, thyroid function tests, urea, creatinine) and urinalysis were in the normal range, and their electrocardiogram was normal. Neither schizophrenic patients nor control subjects suffered from systemic disorders known to be associated with immunological abnormalities. All subjects were free of acute infections or inflammatory reactions at least two weeks before the study.

Clinical assessments

The psychopathological status of each patient was assessed by the same investigator using the Positive and Negative Syndrome Scale (PANSS) (Guy, 1976) and by the Clinical Global Impressions Scale (CGI) in a semi-structured interview. The scores for symptoms included in each PANSS symptom cluster (positive symptoms, negative symptoms, and general psychopathology) were summed.

Pharmacotherapy

Patients were treated with a single neuroleptic as follows: haloperidol (10–20 mg/day, 10 patients), perphenazine (24–48 mg/day, 4 patients), risperidon (4–6 mg/day, 10 patients) seroquel (600–800 mg/day, 7 patients). Biperiden (2–6 mg/day, 21 patients) was administered to alleviate extrapyramidal symptoms. Benzodiazepines were administered as hypnotics (oxazepam 10 mg/day, 10 patients).

Blood sampling

Venous blood was collected into vacutainer tubes. The serum was separated by centrifugation at 300 rpm for 10 min, and the aliquots were stored at –70 °C until assayed. PBMC were isolated from heparinized venous blood by Histopaque-1077 (Sigma-Aldrich, Jerusalem, Israel) gradient centrifugation. The cells were washed twice in phosphate-buffered saline (PBS) and suspended in complete medium (CM).

Ibuprofen

Ibuprofen (Sigma-Aldrich) was freshly dissolved at a concentration of 2 mg/ml in RPMI-1640 medium (Biological Industries, Beith Haemek, Israel) containing 1% penicillin, streptomycin and nystatin, and supplemented with 5% foetal calf serum, designated as CM. Further dilutions were made in CM.

Effect of ibuprofen on cytokine production

2×10^6 PBMC suspended in 1 ml CM were incubated without or with ibuprofen at final concentrations of 20, 100 and 200 μg/ml. Lipopolysaccharide (*E. coli*, LPS, Sigma-Aldrich) was added at the onset of the cultures at a final concentration of 10 ng/ml. The cultures were incubated for 24 h at 37 °C in a humidified atmosphere containing 5 % CO_2. At the end of the incubation period, the culture media were collected, the cells were removed by centrifugation and the supernatants were kept at –70 °C until assayed.

Cytokine content in the supernatants

The concentrations of IL-1β, IL-1ra, IL-6, IL-10 and TNF-α in the supernatants were tested using ELISA kits specific for human cytokines (Biosource International, Camarillo, CA) as detailed in the guideline provided by the manufacturer. The detection level of all cytokines was 30 pg/ml.

Statistical analysis

Statistical analyses were performed using the SPSS 15.0.1 software for Windows®. Data was analysed using ANOVA with repeated measures for each cytokine and for comparison between groups. Paired *t*-test was applied to compare the difference between the level of cytokines obtained with and without various concentrations of ibuprofen. Correlations were analysed using the χ^2 method. The level of significance was set at 5 % ($P < 0.05$) in all analyses.

Results

The mean (± SD) total PANSS score of the schizophrenic patients was 95.88 ± 47.41, the mean (± SD) positive symptoms score was 26.75 ± 76.7, the mean (± SD) negative symptoms score was 29.10 ± 64.0, and the mean (± SD) general psychopathology score was 412.09 ± 48.8. The mean (± SD) Global Assessment Functioning (GAF) scores were 50.00 ± 15.00 and the mean (± SD) CGI scores were 05.0 ± 72.4.

Cytokine production

PBMC from schizophrenic patients and those from the control group incubated with LPS produced similar amounts of IL-1β (6.11 ± 1.0 vs 7.84 ± 0.84 ng/ml; P = 0.224), IL-6 (72.36 ± 5.0 vs 67.2 ± 3.9 ng/ml; P = 0.423), IL-1ra (3.3 ± 0.27 vs 2.56 ± 0.36 ng/ml; P = 0.095) and IL-10 (3.5 ± 0.2 ng/ml vs 3.0 ± 0.22 ng/ml; P = 0.094). However, the production of TNF-α by patients' PBMC incubated with LPS was significantly lower than that secreted by cells from the control group incubated in the same conditions (292 ± 64 vs 429 ± 43 pg/ml, P = 0.049, Tables 1 and 2).

Effect of IB on TNF-α production

Incubation of PBMC from schizophrenic patients and from healthy subjects with increasing concentrations of IB showed significantly enhanced TNF-α secretion ($F3,99 = 29.3$; $P < 0.000$ and $F3,95 = 21.93$; $P < 0.000$, respectively, Table 1). There was a significant difference in the response to IB between the cells from schizophrenic patients and those from healthy individuals ($F1,47 = 6.12$; $P = 0.017$). At IB concentration of 20 µg/ml, PBMC from schizophrenic patients produced lower amounts of TNF-α than cells from the healthy controls ($P = 0.026$), whereas at concentrations of 100 and 200 µg/ml, the production of TNF-α did not differ significantly between the two groups ($P = 0.337$ and $P = 0.911$, respectively). While production of TNF-α by PBMC from healthy subjects cultured with 20, 100 and 200 mg/l of IB was higher by 71 %, 88 % and 52 %, respectively ($P < 0.001$), that of schizophrenic patients increased by 69 % and 49 % at IB doses of 20 and 100 mg/l, respectively ($P < 0.001$), and did not increase at the dose of 200 mg/l.

Effect of IB on IL-6 production

IB induced concentration-dependent stimulation of IL-6 secretion by PBMC from healthy subjects ($F3,95 = 5.964$; $P = 0.001$) and schizophrenic patients ($F3,99 = 7.14$; $P < 0.000$). The production of IL-6 by PBMC from schizophrenic patients with 20, 100 and 200 mg/l of IB increased significantly by 15 %, 31 % and 42 %, respectively ($P < 0.001$), whereas that by cells from the control group increased by 16 % and 23 % at 100 and 200 mg/l, respectively ($P < 0.001$), and did not increase at the dose of 20 mg/l. The difference in response to IB between the two groups was statistically significant ($F1,47 = 4.458$; $P = 0.04$, Table 1).

Effect of IB on IL-1β production

Since IL-1β secretion by human PBMC did not show a Gaussian distribution, statistical analysis of the results was made using log values. The production of IL-1β by PBMC from schizophrenic patients was significantly elevated by increasing concentrations of ibuprofen (F3,99

Table 1. Effect of IB on pro-inflammatory cytokine production by PBMC

Cytokine	Ibuprofen, µg/ml	Healthy adults (N = 24)	Schizophrenic patients (N = 25)	[a]P value
TNF-α, pg/ml	0	429 ± 43	292 ± 64	0.049
	20	725 ± 68***	500 ± 82***	0.026
	100	639 ± 56***	550 ± 99***	0.339
	200	452 ± 44	445 ± 77***	0.911
IL-1β, ng/ml	0	7.84 ± 4.11	6.15 ± 5.36	0.22
	20	9.27 ± 5.51*	6.89 ± 4.69	0.106
	100	8.97 ± 4.28**	7.56 ± 5.48**	0.321
	200	7.91 ± 4.6	7.12 ± 4.96	0.564
IL-6, ng/ml	0	67.20 ± 19.16	72.36 ± 24.95	0.003
	20	71.37 ± 22.19	83.64 ± 31.02**	0.004
	100	77.75 ± 15.16***	94.92 ± 30.27***	0.037
	200	92.41 ± 27.19***	103.12 ± 27.87***	0.305

[a]P values compared between the two groups at the same IB concentration. Asterisks represent a statistically significant difference from LPS-stimulated PBMC of the same group of patients incubated without ibuprofen (*P < 0.05; **P < 0.01; ***P < 0.01).

= 7.144; P < 0.000) whereas that of the control group was not affected significantly (F3,95 = 1.749, P = 0.165). However, there was no significant difference between the two groups concerning the effect of IB on IL-1β production by PBMC (F1,47 = 1.408, P = 0.241, Table 1).

Effect of IB on IL-1ra production

Concentration-dependent inhibition of IL-1ra secretion was observed when PBMC of schizophrenic patients or healthy individuals were incubated with increasing concentrations of IB (F3,99 = 34.043; P < 0.0000 or F3,95 = 19.9; P < 0.0001, respectively, Table 2). At IB concentrations of 100 and 200 mg/l, IL-1ra production by PBMC from healthy subjects was reduced by 14.5 % and 43 %, respectively (P < 0.005), and was not affected by 20 mg/l. Secretion of IL-1ra by cells from schizophrenic patients was modified by IB at a dose of 200 mg/l only (21.5 % reduction, P = 0.006) and not by the other two concentrations. The production of IL-1ra by PBMC in response to IB differed significantly between the two groups (F1,47 = 5.535; P = 0.023).

Effect of IB on IL-10 production

Concentration-related inhibition of IL-10 generation was found when PBMC from schizophrenic patients or healthy subjects were incubated with increasing doses of IB (F3,99 = 139.65, P < 0.0000 or F3,95 = 95.07, P < 0.0000, respectively, Table 2). At IB concentrations of 100 and 200 mg/l, secretion of IL-10 by cells from schizophrenic patients was inhibited by 27 % (P = 0.0014) and 69 % (P < 0.0001), respectively, and that by cells from the control group by 56 % and 85 %, respectively (P < 0.0001). At a lower dose of IB (20 mg/l), secretion of IL-10 by cells from the two groups was not affected significantly. There was a significant difference in the effect of IB on IL-10 production between the two groups (F1,47 = 12.219; P = 0.001).

Correlations between the clinical and immunological parameters

Correlations between the immunological indices and clinical parameters were performed using the Pearson's correlation coefficient. A negative correlation was found between the production of IL-1ra by LPS-stimulated PBMC from schizophrenic patients and the GAF scale (R = –0.413, P = 0.040), and a positive correlation with the general psychopathology subscale score (R = 0.533, P = 0.006). A positive correlation was found between the production of IL-1ra by LPS-stimulated PBMC from schizophrenic patients treated with IB 200 mg/l and total PANSS score (R = –0.421, P = 0.036), and a positive correlation with the general psychopathology subscale score (R = 0.533, P = 0.006). A positive correlation was detected between the production of IL-10 by LPS-stimulated PBMC from schizophrenic patients without and with 100 mg/l IB and the PANSS positive symptoms subscale score (R = –0.432, P = 0.031, R = –0.405, P = 0.044), respectively. There was no correlation between production of different cytokines by LPS-stimulated PBMC with IB and age, illness duration, and CGI.

Discussion

The present results show that the production of IL-1β, IL-6, IL-1ra and IL-10 by LPS-activated PBMC from healthy individuals and schizophrenic patients did not differ significantly, whereas the secretion of TNF-α by cells from schizophrenic patients was significantly lower in comparison to that from healthy subjects. IB caused stimulation of TNF-α production by cells from subjects of the two groups, although cells from schizophrenic patients were less affected. On the other hand, while IB induced stimulation of IL-6 secretion by cells of the two groups, that of the patients was significantly higher. As for IL-1β, the drug caused similar induction of its production by cells from the two groups.

IB inhibited both anti-inflammatory cytokines IL-1ra and IL-10 by cells from the two groups; however, those from healthy volunteers were significantly more affected. Inflammatory reaction is an essential component of the body defence mechanism. Bessler et al. (2002) have reported that 20 μg/ml of IB added to LPS-stimulated PBMC from healthy adults had no effect on IL-1ra secretion. However, at concentrations of 100 and 200 μg/ml, production of this cytokine was significantly suppressed by 11 % and 54 %, respectively. Müller et al. (2002) reported an improvement of psychopathology in schizo-

Table 2. Effect of IB on anti-inflammatory cytokine production

Cytokine	Ibuprofen, μg/ml	Healthy adults (N = 24)	Schizophrenic patients (N = 25)	[a]P value
IL-1ra, ng/ml	0	2.52 ± 1.76	3.29 ± 1.36	0.224
	20	2.41 ± 1.87	3.26 ± 1.34	0.106
	100	2.16 ± 1.84**	3.34 ± 1.44	0.321
	200	1.43 ± 1.63***	2.58 ± 1.11**	0.564
IL-10, ng/ml	0	3.02 ± 1.1	3.53 ± 0.99	0.94
	20	2.76 ± 1.19	3.58 ± 1.03	0.014
	100	1.32 ± 0.97***	2.59 ± 0.96**	0.001
	200	0.45 ± 0.26***	1.10 ± 0.70***	0.001

[a]P values compared between the two groups at the same IB concentration. Asterisks represent a statistically significant difference from LPS-stimulated PBMC of the same group of patients incubated without ibuprofen (*P < 0.05; **P < 0.01; ***P < 0.01).

phrenic patients treated with celecoxib compared to placebo, but only in patients with an illness duration of < 2 years. The balanced synthesis and release of pro-inflammatory cytokines and their natural inhibitors play a pivotal role in pathogenesis of inflammatory diseases. The present results suggest that while in cells from healthy adults IB interferes with the delicate balance between pro- and anti-inflammatory cytokines, this effect is not apparent in the cells from schizophrenic patients. Our findings reveal a lower ability of PBMC from schizophrenic patients to produce IL-1ra in comparison to cells from healthy adults.

This is in line with other reports (Sirota et al., 1995, 2005) indicating a reduced capacity of schizophrenic patients' cells to produce pro- and anti-inflammatory cytokines, supporting the existence of a flaw in their immune system. A significant positive correlation was found between the high serum IL-1ra delta (baseline values minus end line values) level and delta PANSS negative symptoms score, indicating negative psychopathology scores and supporting the existence of a link between ongoing psychotic episodes, immunological insult and immuno-neutralization induced by IL-1ra (Maier et al., 1995). In a previous study we have found that the serum level of IL-1ra was increased in a small subgroup of neuroleptic-naïve schizophrenic patients at baseline and in a total group of schizophrenic patients after eight weeks of neuroleptic treatment as compared with the level in healthy controls (Sirota et al., 2005).

Activation of the monocytic arm of cell-mediated immunity in schizophrenic patients is expressed by increased concentrations of IL-1β in the serum and in cultured mononuclear cell supernatants, and increased plasma IL-1ra and IL-6 concentrations (Naudin et al., 1997; Akiyama., 1999). The increased secretion of IL-1ra and IL-6 by LPS-stimulated PBMC of schizophrenic patients found in the present study is in accordance with these reports. Since lower levels of IL-1ra mRNA have been found in the prefrontal cortex of schizophrenic patients, it has been suggested that chronic schizophrenia is characterized by down-regulation of IL-1ra production in the prefrontal region, reflecting an immunopathological trait in this area, irrespective of its impact on the periphery (Toyooka et al., 2003). It is notable that IL-10 is an anti-inflammatory cytokine that not only down-regulates secretion of pro-inflammatory cytokines, but induces secretion of anti-inflammatory cytokines, potentially leading to reduction of chronic inflammation in schizophrenia (Kunz et al., 2011). Potvin et al. (2008) conducted a meta-analysis of 62 cross-sectional studies of plasma or serum cytokine concentrations *in vivo* and secretion of cytokines by peripheral blood leukocytes *in vitro* from 2298 schizophrenic patients and 1858 healthy volunteers. The results showed that in schizophrenic patients, there is an increase in serum IL-1ra, sIL-2r and IL-6 and a decrease of IL-2 production *in vitro*. No significant effects were obtained for IFN-γ, IL-4, IL-1β, TNF-α, sIL-6r, and IL-10.

These findings support the existence of an abeyant inflammatory syndrome in schizophrenia. Interestingly, IL-1ra and IL-6 are predominantly produced by cells of the innate immunity, suggesting primary alterations of this arm of the immune system in schizophrenic patients. The present study encompasses certain limitations. Thus, stress that schizophrenic patients are sensitive and known to trigger psychotic relapses by hormonal and immunological mechanisms may induce elevation of the peripheral inflammatory cytokine level (Müller, 2014). Furthermore, cytokine alterations in schizophrenia might be secondary to weight gain and obesity (Sirota et al., 2015), influenced by poor dietary conditions, sedentary lifestyles and especially second-generation antipsychotic drugs that have the potential to exert an impact on the immune system. This point has raised a certain scepticism about the concept that schizophrenia is as an autoimmune disorder. Since our patients were treated with different antipsychotic drugs, their effect on the results cannot be excluded. It is notable that a meta-analysis on the association between several infectious agents and schizophrenia showed a statistically significant association between schizophrenia and infections (Müller, 2014). It has been reported that the risk for development of schizophrenia increases with an incidence rate ratio of 3.40 when three or more infections and an autoimmune disease are associated. These results remained significant after adjusting for substance use disorders and history of family psychiatric disorders.

In short, the results of the study show that LPS-stimulated PBMC from schizophrenic patients differ from those of healthy subjects in their ability to produce TNF-α but secrete similar amounts of IL-1β, IL-6, IL-1ra and IL-10. Moreover, the stimulation of TNF-α and IL-6 production caused by IB was affected differently by cells from schizophrenic patients as compared to controls. IB inhibited IL-1ra and IL-10 production by cells from the two groups, although those from healthy volunteers were more affected. The activation of PBMC by ibuprofen suggests that the drug may exert a beneficial effect on schizophrenic patients.

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Authors' contribution: P. S. conceived the basic idea of the study. D. C. T. was engaged with the clinical and therapeutic status of the patients including obtaining blood samples. H. B. performed and analysed the laboratory data. All authors participated in interpretation of the results, collection of relevant literature and drafting of the ms. M. D. reviewed the references and wrote the final version of the ms. All authors agreed with the final style of the study and its submission for publication.

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