Increased serum interleukin-1β and interleukin-6 in elderly, chronic schizophrenic patients on stable antipsychotic medication

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Abstract: In schizophrenia, alterations of proinflammatory cytokine levels have been reported and related to the disease and psychopathology. However, only limited conclusions can be drawn in view of confounding factors such as infection, age, sex, smoking, and antipsychotic medication. Chronic schizophrenic patients with a long-term disease process and medication period have not been investigated so far. We have measured serum levels of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)α in 41 elderly, chronic schizophrenic patients and 23 age- and sex-matched controls using enzyme-linked immunosorbent assay (ELISA). We assessed detailed psychopathology and neuropsychological performance and determined serum levels of haloperidol, clozapine, and the two main clozapine metabolites, desmethylclozapine and clozapine metabolite *N*-oxide, by high-pressure liquid chromatography (HPLC). IL-1β and IL-6 levels were increased in treatment-resistant schizophrenic patients compared with healthy controls, whereas TNFα showed no difference. We did not find statistically significant differences of cytokine levels between medication groups and there was no correlation with serum levels of antipsychotics or psychopathological rating scores. Elevations of IL-1β and IL-6 in elderly chronic schizophrenic patients may be related to an active disease process lasting until old age. Despite missing correlations, long-term treatment effects in treatment-resistant patients may have affected TNFα, leading to control levels. Post-mortem and animal studies should clarify the presence of altered immune function in the brain as well as the effect of cytokine levels in relation to neurodevelopmental disturbances and schizophrenia-associated behavior.

Keywords: interleukin-1β, interleukin-6, TNFα, schizophrenia, haloperidol, clozapine

Introduction

Schizophrenia is a severe psychiatric disorder with positive symptoms such as hallucinations, thought disorder, and delusions as well as negative symptoms. During recent decades, evidence for the involvement of the immune system has accumulated. Schizophrenia has been associated with decreased mitogen-induced lymphocyte proliferation (Chengappa et al 1995), increased numbers of total T and T-helper cells (Muller et al 1993), and the presence of antibrain antibodies in serum (Henneberg et al 1994). However, the pathophysiology of the disease remains elusive.

Cytokines are proteins that modulate systemic and central nervous system (CNS) responses to infection, inflammation, and injury (Rothwell 1999). They are released from various blood cells (macrophages, monocytes, T and B cells). In the brain they are expressed by neurons, astroglia, and microglia and regulate brain development (Kreutzberg 2000; Vitkovic et al 2000). Proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)α may play an important role in the

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association between prenatal virus infection and schizophrenia (Nawa et al 2000; Urakubo et al 2001).

Changes in cytokines and cytokine receptors have been reported in plasma, serum, and cerebrospinal fluid (CSF) of schizophrenic patients, such as increased IL-6, IL-1β, and TNFα (Shintani et al 1991; Ganguli et al 1994; Xu et al 1994; Maes et al 1995; Naudin et al 1996; Frommberger et al 1997; Monteleone et al 1997; Lin et al 1998; van Kammen et al 1999; Theodoropoulou et al 2001; Zhang et al 2002; Garver et al 2003). However, results are inconsistent (Baker et al 1996; Kim et al 1998; Haack et al 1999; Kudoh et al 2001), but several investigations have shown a relationship between IL-6 and negative symptoms, duration of the disease (Ganguli et al 1994; Akiyama 1999; Kim et al 2000), acute state of the disorder (Frommberger et al 1997), and treatment resistance (Lin et al 1998). These findings suggest that high IL-6 levels are associated with an unfavorable course of the disease (Müller et al 2000). On the other hand, IL-6 levels have been reported to be higher in younger schizophrenic patients than in older individuals (Maes et al 1994). So far, hospitalized elderly patients with long-term schizophrenia and persistent positive and negative symptoms as well as neurocognitive deficits have not been investigated. We hypothesize that chronic elderly schizophrenic patients have elevated serum levels of IL-6, IL-1β, and TNFα compared with age-matched healthy controls.

However, factors such as age, body mass index, sex, smoking habits, recent infectious diseases, and medication have been reported to affect IL-6 levels (Haack et al 1999). Repeated blood drawings by intravenous catheter, but not by needle stick, increased local IL-6 levels (Haack et al 2002). In CNS cell cultures, typical neuroleptics such as phenothiazines inhibited production of IL-6 after stimulation with polysaccharides (Müller et al 2000). Haloperidol normalized the increased serum IL-6 levels in acute schizophrenic patients (Maes et al 1994, 1995, 1997). Additionally, haloperidol decreased IL-1β and TNFα released from monocytes (Kowalski et al 2001). In contrast, clozapine stimulated in vivo release of IL-6 and TNFα during the first weeks of treatment (Pollmacher et al 1996; Maes et al 1997; Hinze-Selch et al 2000). After 10 weeks of clozapine administration, TNFα levels have been reported to have decreased (Monteleone et al 1997). Regarding plasma levels of the IL-1β receptor antagonist, there are conflicting results (Pollmacher et al 1996; Maes et al 1997). However, long-term effects of typical and atypical antipsychotic treatment on circulating cytokine levels have not yet been investigated. In addition, effects of clozapine metabolites on serum cytokines are unknown. To address this, we investigated serum IL-1β, IL-6, and TNFα levels, psychopathological and neuropsychological scores, and haloperidol, clozapine, and clozapine metabolite levels in elderly, chronic schizophrenic patients with persisting symptoms and stable long-term typical neuroleptic and clozapine treatment.

Methods

Subjects

The study was approved by the Ethics Committee of the Clinic Mannheim, Heidelberg University. We investigated 41 schizophrenic patients (24 men, 17 women, mean age 63.3 ± 7.0 years) who met the DSM-IV diagnostic criteria for chronic schizophrenia (APA 1994) and 23 age- and sex-matched healthy controls (15 men, 8 women, mean age 64.5 ± 9.8 years). Neither the schizophrenic patients nor the healthy controls suffered from substance or alcohol abuse, systemic disorders known to be associated with immunological abnormalities, nor were they receiving immunosuppressive drugs. This was determined by anamnesis, clinical examination, and routine blood tests (including white blood cell differentiation, liver parameters, electrolytes, and blood sedimentation rate). Twenty-one schizophrenic patients and six healthy controls were smokers.

In schizophrenic patients, duration of the illness was 35.3 ± 11.4 years, age at onset was 28.0 ± 10.7 years, and duration of antipsychotic medication was 27.2 ± 12.3 years. Mean chlorpromazine equivalents (CPE) at the time of blood collection were 708.5 ± 591.7 mg/day (Jahn and Mussgay 1989; Meltzer and Fatemi 1998). Fourteen schizophrenic patients were treated with haloperidol and additive typical neuroleptics such as perazine, chlorprothixene, thioridazine, and promethazine (CPE 1302.8 ± 1253.0 mg/day). Fifteen patients were treated solely with clozapine (CPE 316.5 ± 111.0 mg/day), and eight patients received a combination of haloperidol, typical neuroleptics, and clozapine (CPE 585.7 ± 382.3 mg/day).

Psychopathological and neuropsychological assessments

Dementia in patients and healthy controls was evaluated using the Mini-Mental State Examination (MMSE) (Folstein et al 1975). The psychopathological status of schizophrenic patients was assessed by a trained physician using the Scale for Assessment of Positive Symptoms (SAPS) (Andreasen

1983a), the Scale for Assessment of Negative Symptoms (SANS) (Andreasen 1983b), and the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962). Depressive symptoms were assessed by Hamilton Depression Rating Scale (HAM-D) (Hamilton 1960).

Cytokine measurements in serum

Sera from patients and controls were collected between 9 am and 10 am by single needle stick and stored at –80 °C until thawed for assay. IL-1β, IL-6, and TNFα were measured in one sample with highly sensitive enzyme-linked immunosorbent assay (ELISA) kits (R&D Diagnostics, Wiesbaden, Germany) specific for the human cytokines according to the manufacturer's instruction. Cytokine assays for each patient and his/her matched control were run in the same lot.

Determination of serum levels of haloperidol and clozapine

Sample preparation was performed by liquid–liquid extraction: to 1 mL serum, 2 mL ethyl acetate (supplemented with 3% ammonium hydroxide) and 40 ng of chlorinated haloperidol (as internal standard) were added. Samples were agitated for 30 minutes at 4 °C and then centrifuged at 1850 *g* for 15 minutes. The organic supernatant was collected and concentrated using a SpeedVac-Plus concentrator (SC 110, Savant, USA). The residue was resolved in 0.2 mL mobile phase and 80 µL was injected into the chromatograph. Calibration standards were prepared in serum using concentrations of 50, 100, 200, 400, 600, and 800 ng/mL for clozapine and its desmethyl metabolite. Concentrations of 12.5, 25, 50, 100, 200, and 400 ng/mL for clozapine *N*-oxide metabolite and 1.25, 2.5, 5, 10, 20, and 40 ng/mL for haloperidol were used.

As eluent in high-pressure liquid chromatography (HPLC), an acetonitrile-ammonium-acetate buffer (80:20%, v/v) supplemented with 0.83 mmol/L 1-octanesulfonic acid sodium salt monohydrate was prepared. The pH value was adjusted to 4.5 using orthophosphoric acid (85%). Isocratic separation was performed at 1 mL/min and 37 °C. Reversed-phase HPLC with ion-pair chromatography was performed with a stainless-steel column (250 mm × 4.6 mm ID) packed with Hypersil CPS, 5 µm (MZ, Germany). As guard column the Security-Guard-System (Phenomenex, Germany) was used. Effluent was monitored at 254 nm with a variable-wavelength UV detector (Lambda-max, model 481, LC spectrometer, Waters, Germany). Analysis was performed using a Dionex instrumentation setting composed of a model P 580 pump and a GINA 50 autosampler (Dionex, Germany). Chromatograms were recorded and analyzed using the Chromeleon integration software (Dionex, Germany).

All chemicals and drugs were delivered from Sigma-Aldrich (Germany).

Statistics

Graphical inspection of the data by histograms and results of Kolmogorov-Smirnov tests showed the non-normal distribution of the data. Thus, comparisons between schizophrenic patients and healthy controls as well as between different treatment groups were carried out by the nonparametric Mann-Whitney U-test using Bonferroni correction. Correlations between the variables were analyzed by Spearman correlation coefficients. Statistical tests were performed using SPSS 10.0. Results are expressed as mean ± standard deviation.

Results

Serum IL-1β ($p < 0.05$) and IL-6 ($p < 0.05$) levels were increased in all schizophrenic patients compared with healthy controls, whereas TNFα showed no difference (Figure 1). Serum cytokine levels did not differ in smokers compared with non-smokers. Additionally, cytokine levels were not different between treatment groups. No significant correlations have been found between age and cytokine levels.

In the haloperidol group, mean serum haloperidol level was 29.3 ± 36.5 ng/mL; in the clozapine group, mean levels were clozapine 351.3 ± 254.2 ng/mL, desmethylclozapine 182.9 ± 120.7 ng/mL, and the clozapine breakdown product

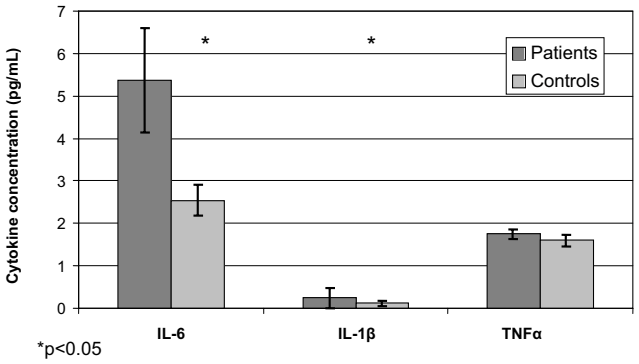

Figure 1 Serum interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)α in elderly chronic schizophrenic patients compared with healthy controls. IL-6 and IL-1β were significantly increased in schizophrenic patients ($p < 0.05$). Variables are expressed as mean ± standard deviation.

N-oxide 143.5 ± 157.1 ng/mL. In the group with combined medication, mean levels were as follows: haloperidol 25.9 ± 22.8 ng/mL, clozapine 429.9 ± 315.4 ng/mL, desmethylclozapine 326.9 ± 192.6 ng/mL, and clozapine metabolite *N*-oxide 168.2 ± 96.9 ng/mL. IL-6, IL-1β, and TNFα levels did not correlate with serum haloperidol, clozapine, desmethylclozapine, or clozapine metabolite *N*-oxide levels.

In healthy controls, dementia was excluded, and mean MMSE score was 29.4 ± 0.6. In schizophrenic patients, scores were as follows: MMSE 23.8 ± 6, BPRS 46.2 ± 14.9, SAPS 44.3 ± 27.9, SANS 45.6 ± 22.7, and HAM-D 9.9 ± 6.4. MMSE and psychopathological scores revealed no correlations with cytokine levels. In schizophrenic patients, MMSE scores correlated significantly negatively with levels of haloperidol ($p < 0.05$, correlation coefficient –0.616), clozapine ($p < 0.05$, correlation coefficient –0.554), and clozapine metabolite *N*-oxide ($p < 0.05$, correlation coefficient –0.603).

Discussion

This study revealed significant differences in serum levels of IL-1β and IL-6 in elderly, chronic schizophrenic patients compared with age- and sex-matched healthy controls. Our results confirm recent studies reporting increased IL-1β and IL-6 levels in younger first-episode and chronic schizophrenic patients (Ganguli et al 1994; Katila et al 1994; Maes et al 1994, 1995; Naudin et al 1996; Frommberger et al 1997; Lin et al 1998; Akiyama 1999; van Kammen et al 1999; Theodoropoulou et al 2001; Zhang et al 2002) and support the hypothesis of disturbed immune function in schizophrenia lasting until old age. However, the aforementioned studies differ in methodology (eg, plasma vs serum), and previous studies did not address age and medication effects or smoking habits.

Age has been shown to induce increasing activity of the TNFα and IL-6 system (Haack et al 1999). In contrast, IL-6 levels have been reported to be elevated in younger schizophrenic patients, but not in patients older than 35 years (Maes et al 1994). In our study, we did not find a correlation between age and cytokine levels in elderly schizophrenic patients or healthy controls, and age did not differ between groups. Thus, the correlation between age and IL-6 levels in elderly subjects is questionable. However, only elderly patients were included in the sample. In order to find a correlation, a sufficient degree of age variance is necessary, which may not be present in our selected sample.

In our study, schizophrenic patients had lower MMSE scores than healthy controls. Thus, differences in cytokine levels may be affected by coexisting dementia. However, MMSE scores correlated negatively with serum levels of antipsychotics and may be influenced by medication effects such as sedation. Post-mortem studies of plaques, tangles, and cytokine levels may elucidate the correlation between dementia and cytokines.

Despite the absence of significant correlations between psychopathology and cytokine levels, state of the illness may have an influence on cytokine levels, since all our patients had a chronic course of the disorder. IL-6 levels have been reported to be higher during the acute state of the disease and to decline after remission (Naudin et al 1996; Frommberger et al 1997). Additionally, elevated IL-1β levels have been reported in acute but not in chronic schizophrenic patients (Katila et al 1994). Our study sample consisted of chronic patients with persisting positive and negative symptoms as well as neurocognitive deficits. Thus, our results in chronic patients confirm higher serum IL-6 levels in treatment-resistant schizophrenic patients than in healthy controls and patients without treatment resistance (Lin et al 1998). However, in our elderly study group with persisting symptoms and long-term duration of the disease we did not investigate treatment resistance. Nor did we confirm correlations between IL-6 levels and negative symptoms or correlation with duration of illness measured in younger schizophrenic patients (Ganguli et al 1994; Kim et al 2001).

Sex-related differences found previously in psychiatric patients, such as higher TNFα levels in women than in men (Baker et al 1996) and higher IL-6 levels in males (Ganguli et al 1994), could not be confirmed by our study. In addition, we did not find higher IL-6 levels in males than in females. In accordance with other studies in medicated chronic schizophrenia (Naudin et al 1996), we did not confirm elevated TNFα levels in mostly unmedicated schizophrenic patients compared with healthy controls (Monteleone et al 1997; Theodoropoulou et al 2001).

Although differences have not been detected between patients on and off neuroleptic medication (Katila et al 1994; Theodoropoulou et al 2001), treatment effects may have affected TNFα in our patients, leading to control levels. Clozapine has been reported to decrease TNFα to normal levels without effects on IL-6 after a 10-week treatment period (Monteleone et al 1997). In contrast, short-term clozapine treatment has been shown to increase TNFα levels (Pollmacher et al 1996; Hinze-Selch et al 2000).

Additionally, typical neuroleptics such as haloperidol and perazine decreased the release of TNFα and IL-1β from monocytes to control levels (Kowalski et al 2001). Inhibitory effects of chlorpromazine on TNFα production have also been observed (Bertini et al 1993). Thus, normal TNFα levels of schizophrenic patients in our study may be the consequence of long-term treatment with clozapine and typical neuroleptics. However, there was no correlation of serum clozapine, clozapine metabolite, and haloperidol levels with IL-1β or TNFα. Furthermore, we did not detect differences in IL-6 and IL-1β levels between treatment groups, suggesting no specific long-term treatment effects on these levels. However, in our naturalistic study design, patients were not treated with haloperidol as monotherapy, and other typical neuroleptics such as perazine, chlorprothixene, thioridazine, and promethazine could have influenced cytokine levels. Thus, additional studies using monotherapy are warranted.

Clozapine has been shown to increase plasma IL-6 levels only in vitro and after 2 weeks of treatment, but not during longer treatment periods (Maes et al 1994, 1997; Hinze-Selch et al 1998). Thus, clozapine may have short-lasting immunomodulatory effects that are associated with clozapine-induced fever and agranulocytosis (Pollmacher et al 2000). Our results suggest that effects of long-term clozapine treatment differ from short-term effects. In contrast, our results confirm previous studies reporting no effects of haloperidol treatment on IL-6 levels (Kim 1986; Pollmacher et al 1997). In addition, we did not detect effects of smoking on proinflammatory cytokines and did not confirm a study of smoking effects on IL-6 levels (Haack et al 1999).

Elevated IL-6 plasma and CSF levels have been shown in patients with autoimmune diseases (Hirano et al 1996) and inflammation of the CNS (Hirohata et al 1993). Both IL-1β and IL-6 have been reported to exert an effect on neuronal growth and differentiation (Giulian et al 1988; Hama et al 1991). However, it is still controversial whether peripheral cytokines act in the brain, because cytokines do not easily cross the blood–brain barrier (Hopkins and Rothwell 1995; Rothwell and Hopkins 1995). Cytokines may actively be transported into the brain or may be produced in the brain itself. They may also act indirectly on the CNS by activation of endothelial cells and the autonomic system to stimulate their own synthesis in the brain (Pollmacher et al 2000). In addition, IL-6 is known to disturb the blood–brain barrier function (Frei et al 1989). Polymorphisms in TNFα and IL-1β genes have been associated with schizophrenia (Boin et al 2001; Schwab et al 2003; Tan et al 2003; Zanardini et al 2003; Rosa et al 2004) and may contribute to altered protein levels in brain and periphery and affect brain morphology (Meisenzahl et al 2001), but results are still contradictory (Tatsumi et al 1997; Riedel et al 2002; Handoko et al 2003; Tsai et al 2003; Yang et al 2003).

To date, there is only one post-mortem study reporting decreased gene and protein expression of the IL-1β inhibiting IL-1 receptor antagonist, but no diagnosis effects on IL-1β expression in the prefrontal cortex of schizophrenic patients (Toyooka et al 2003). Since microglia produces cytokines upon activation (Kreutzberg 2000) and microglial dysfunction may be involved in the pathophysiology of schizophrenia (Munn 2000; Radewicz et al 2000), gene and protein expression studies in post-mortem brains are warranted to elucidate regulation of cytokines in the CNS.

Expression and signaling of cytokines may be influenced by environmental factors such as stress, infection, and brain activity (Nawa et al 2000). A number of environmental factors have been implicated in schizophrenia, including hypoxia (Cannon et al 2002; Van Erp et al 2002), maternal viral infection (Buka et al 2001), nutritional deficiency (Susser et al 1996), and season of birth (Pulver et al 1981). All of these could influence cytokine levels. Proinflammatory cytokines are increased during CNS ischemia (Saito et al 1996; Orzylowska et al 1999). Season of birth, however, has no apparent effects on cytokine levels in schizophrenia (Altamura et al 2003). By contrast, in keeping with the neurodevelopmental hypothesis, animal studies revealed schizophrenia-related behavioral abnormalities such as latent inhibition in adult animals after immune activation during pregnancy (Zuckerman et al 2003). In embryonic cell culture, IL-1β and IL-6 induced decreases in the number of neurons immunoreactive for microtubule-associated protein 2, suggesting decreased neuronal survival (Marx et al 2001). Moreover, cytokines are known to influence behavior such as food and water intake, social interaction, learning and cognitive function, sexual behavior, sleep, and anhedonia (Larson and Dunn 2001). Comedication of schizophrenic patients with the cyclo-oxygenase-2 inhibitor celecoxib showed greater improvement in psychopathological scores than antipsychotic treatment alone, suggesting that immune dysfunction in schizophrenia is somehow linked to the pathomechanisms of the disorder (Muller et al 2002). Thus,

increased IL-1β and IL-6 levels may be associated with schizophrenia-related behavior. Further neurodevelopmental animal studies could clarify the relationship between adult cytokine levels and schizophrenia-associated behavior.

In summary, elevated IL-1β and IL-6 levels may be related to chronic schizophrenia even in old age. Confounding factors such as age, sex, smoking, and antipsychotic medication have not been shown to affect these serum cytokine levels. However, we have investigated only a small sample size related to the quantity of confounding variables, and correlations may become significant in a larger sample. In addition, some factors such as additional treatment with benzodiazepines or anticholinergic medication have not been investigated. Further studies should investigate cytokine expression in post-mortem brains. To clarify the effect of neurodevelopmental disturbances on immune function and schizophrenia-related behavior, investigations of animal models are warranted.

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