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Cortisol and Cytokines in Chronic and Treatment-Resistant Patients with Schizophrenia: Association with Psychopathology and Response to Antipsychotics

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The bilateral communication between the immune and neuroendocrine systems plays an essential role in modulating the adequate response of the hypothalamic–pituitary–adrenal (HPA) axis to the stimulatory influence of cytokines and stress-related mediators. Growing evidence suggests that neuro-immune-endocrine crosstalk may be impaired in schizophrenia. We determined the relationship between cortisol, cytokines interleukin-2 (IL-2) and interleukin-6 (IL-6), and symptoms in schizophrenia during treatment with typical and atypical antipsychotic drugs. Subjects included 30 healthy controls (HC) and 78 schizophrenic (SCH) in-patients. SCH were randomly assigned to 12-week treatment with 6 mg/day of risperidone or 20 mg/day of haloperidol using a double-blind design. Clinical efficacy was determined using the Positive and Negative Syndrome Scale (PANSS). Serum cortisol and IL-2 levels were assayed by radioimmunometric assay, and serum IL-6 levels by quantitative enzyme-linked immunosorbent assay. Following a 2-week washout period, serum levels of cortisol, IL-2, and IL-6 were increased in patients with schizophrenia compared to HC. Elevations in cortisol were associated with increase in both IL-2 and IL-6 in SCH. Moreover, elevations in cortisol were associated with negative symptoms and IL-2 with positive symptoms. In all, 12 weeks of risperidone treatment significantly decreased elevated cortisol and improved negative symptoms, but produced similar effects on IL-2 and IL-6 as well as on positive symptoms compared to haloperidol. The improvement of negative symptoms was related to the change in cortisol. Our results suggest that the imbalance in the HPA axis and cytokine system in patients with SCH is implicated in clinical symptoms, and is improved with atypical antipsychotic treatment.
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INTRODUCTION

There is a complex bidirectional communication between the nervous, endocrine, and immune systems that can be demonstrated by the presence of shared neurotransmitters, hormones, and cytokines (Blalock, 1989; Haddad *et al*, 2002). Communication between these systems plays an essential role in modulating the adequate response of the hypothalamic–pituitary–adrenal (HPA) axis to the stimulatory influence of cytokines and stress-related mediators (Spangelo *et al*, 1995). Growing evidence suggests that, in addition to providing communication between immune cells, specific cytokines play a role in signaling the brain to produce neurochemical, neuroendocrine, neuroimmune, and behavioral changes (Muller and Ackenheil, 1998; Kronfol and Remick, 2000). Recently, studies have shown that the interface between these complex systems is impaired in schizophrenia (SCH, Altamura *et al*, 1999). Specially, SCH has been associated with several immunological abnormalities, including decreased mitogen-induced lymphocyte proliferation (Chengappa *et al*, 1995), altered numbers of total T and T-helper cells (Muller *et al*, 1993), the presence of antibrain antibodies in serum (Henneberg *et al*, 1994), and changes in cytokines and cytokine receptors in the blood and the cerebrospinal fluid (CSF) (Licinio *et al*, 1993; Rapaport *et al*, 1994, 1997; Ganguli *et al*, 1994, 1995; Maes *et al*, 1995, 1997, 2000, 2002; Lin *et al*, 1998; Arolt *et al*, 2000; Zhang *et al*, 2002a, b). Together, these studies show that SCH is associated with significant immunological alterations, and activation of the

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inflammatory response system (IRS) is mediated, in part, by cytokines. Interestingly, these abnormalities are particularly prominent in treatment-resistant SCH (Lin *et al*, 1998; Maes *et al*, 2000, 2002).

Evidence also suggests dysfunction in the HPA axis in SCH (Walker and Diforio, 1997; Marx and Lieberman, 1998). Abnormalities in the HPA axis of SCH have been shown using the dexamethasone suppression test (DST). Nonsuppression, due to the lack of glucocorticoid secretion feedback mechanisms, occurs frequently in SCH, with percentages varying between 11 and 55% (Sharma *et al*, 1988; Yeragani, 1990; Coryell and Tsuang, 1992). Moreover, several studies showed that basal cortisol levels are significantly higher in schizophrenic (SCH) patients compared to normal controls (Walker and Diforio, 1997; Lammers *et al*, 1995), although these findings have not been consistent between studies (Jansen *et al*, 1998; Kaneda *et al*, 2002). Studies also suggest a relationship between HPA activity and symptomatology in SCH. Cortisol secretion has been associated with more severe positive symptoms (Kaneko *et al*, 1992; Walder *et al*, 2000), whereas in others it was associated with higher ratings of negative symptoms (Newcomer *et al*, 1991; Tandon *et al*, 1991). Some investigators purport that in SCH, there is an association of DST nonsuppression with negative symptoms (Altamura *et al*, 1989; Newcomer *et al*, 1991; Tandon *et al*, 1991). Taken together, these results suggest that HPA axis dysregulation/activation and hypercortisolemia are frequent present in SCH patients.

Wang and Dunn (1999) reported that HPA axis activation can be elicited by exogenous cytokines, such as interleukin-1 (IL-1), IL-6 when administered to rodents. Several cytokines are also known to affect the release of anterior pituitary hormones by an action on the hypothalamus and/or the pituitary glands (Bumiller *et al*, 1999). Moreover, as mentioned previously, SCH is associated with a hyperresponsive IRS. Therefore, Altamura *et al* (1999) proposed the hypothesis that hypercortisolemia observed in SCH could be induced by cytokines. So far, however, this association remains to be elucidated.

In view of the previously mentioned studies and the close relationship between HPA axis and immune system, we tested the hypothesis that alterations in neuroendocrine systems may be related to changes in immune system in SCH; changes that could be especially apparent in treatment-resistant SCH. Also, we speculate that inappropriate interaction of HPA axis and cytokines might play a role in the pathogenesis of some symptoms occurring in SCH. Further, we wanted to determine if typical or atypical antipsychotics-induced effects on HPA axis and IRS could account for the differences in clinical response, which, to our knowledge, has not been investigated so far. Therefore, the purpose of the study was to investigate whether (1) serum cortisol, IL-2, and IL-6 were increased simultaneously in chronic and treatment-resistant schizophrenic patients; (2) there was a relation between cortisol and cytokines, or between symptom severity and both cortisol and cytokines; (3) there was a significant difference between typical and atypical antipsychotic drugs in the influence on the serum cortisol, IL-2, and IL-6; (4) there were any relationships between the changes of serum cortisol, IL-2, and IL-6 and the changes of psychopathological symptoms.

METHODS AND SUBJECTS

Subjects

Physically healthy Chinese in-patients were diagnosed as meeting the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R) criteria for SCH, using the Structured Clinical Interview for DSM-III-R (SCID). All patients were considered resistant to treatment if meeting the following criteria: no response to at least three antipsychotics treated 3 months or over at full dose, equivalent to chlorpromazine 800 mg/day. Both patients and 30 normal volunteers were recruited at the same period from Beijing area. Normal controls were recruited from the community, and matched for age and gender. Both patients and matched normal subjects had similar socioeconomic status. Demographic data for patients and normal controls are summarized in Table 1.

All subjects gave informed written consent to participate in the study, which was approved by the Institutional Review Board, the Institute of Mental Health, Peking University. They were screened with a complete physical, neurological, and psychiatric evaluation conducted by the clinical physicians. None of the SCH patients nor control subjects suffered from substance abuse/dependence or were receiving immunosuppressive drugs.

Clinical Treatment and Clinical Ratings

Clinical treatment and ratings have been described in detail in our earlier report (Zhang *et al*, 2001). Briefly, the clinical trials consisted of a 2-week placebo lead-in followed by 12 weeks of double-blind treatment. Medications that patients had been taking prior to the 2-week washout period were either clozapine ($n=20$), perphenazine ($n=17$), chlorpromazine ($n=16$), haloperidol ($n=15$), thioridazine ($n=6$), or sulpiride ($n=4$). A total of 78 patients were randomized into risperidone group ($n=41$) and haloperidol group ($n=37$). The dose of risperidone was increased to 6 mg/day and the dose of haloperidol to 20 mg/day during the first week of blind administration, and the doses were maintained at those levels until the end of the trial.

The Positive and Negative Syndrome Scale (PANSS) (Kay *et al*, 1987) was administered by four clinical psychiatrists who were blind to the treatment. To ensure consistency and

Table 1 Demographics of Patients and Normal Control Subjects

	Risperidone ($n=41$)	Haloperidol ($n=37$)	Control subjects ($n=30$)
Sex, M/F	30/11	30/7	22/8
Age (years)	43.8±6.4	43.7±8.1	40.4±10.3
Duration of illness (years)	21.6±10.9	19.2±9.4	NA
Smokers	28 (68%)	24 (65%)	19 (63%)
Smoked cigarettes	12.4±8.3	12.1±8.1	11.9±5.0

All data were reported as mean±SD unless otherwise indicated. There were no significant differences among risperidone, haloperidol and control groups on any characteristic by χ^2 test and analysis of variance (ANOVA), followed by *post hoc* tests (Fisher's LSD test).

reliability of ratings across study, four psychiatrists attended a training session in the proper use of the PANSS before study began. After training, a correlation coefficient greater than 0.8 was maintained for the PANSS total score by repeated assessments.

Cortisol Assay

Serum samples of patients were collected between 0700 and 0900 h at the end of the 2-week washout period and after 12-week treatment. Samples from healthy controls were collected at the same period as that of the patients at the end of washout period. The serum was separated via centrifugation, aliquoted and stored at -70°C until assayed.

Serum cortisol was determined by radioimmunometric assay (RIA). The assay was performed in accordance with the manufacturer's instructions (Tianjin DePu Inc., China). Each sample was run in duplicate. The sensitivity was 1 ng/ml, intra- and inter-assay variation coefficients were 6 and 10%, respectively.

Measurement of Serum IL-2 and IL-6

IL-2 was assayed in serum by RIA (ChangZheng Inc., Chinese Second Military Medical University, Shanghai, China). The assay was performed in accordance with the manufacturer's instructions. Each assay was run in duplicate. The sensitivity was 0.2 ng/ml, intra- and inter-assay variation coefficients were 6 and 8%, respectively.

Serum IL-6 levels were measured in duplicate by sandwich ELISA using a commercially available kit (BanDing Biological Inc., Chinese Academy of Sciences, Beijing, China). The sensitivities were 0.2 ng/ml, and intra- and inter-assay variation coefficients were 7 and 9%, respectively.

All samples were assayed by the same investigator who was blind to the treatment condition.

Statistical Analyses

Since the majority of the interleukin variables were not normally distributed in patients (Kolmogorov–Smirnov one-sample test), and although all these variables were normally distributed in normal controls, the principal analysis consisted of nonparametric tests for comparison between groups of patient and normal control (Mann–Whitney). Since serum cortisol was normally distributed in both patients and normal controls, analysis of variance (ANOVA) was used. Analysis of covariance (ANCOVA) was also performed, using age, duration of illness, baseline cortisol level, baseline PANSS score as covariates, followed by Fisher's least significant difference (LSD) test to compare the differences in cortisol levels between the groups. Correlation among cortisol, IL-2, and IL-6 levels and clinical ratings were examined by bivariate correlation (Spearman or Pearson correlation coefficients). Where there was a significance, the Bonferroni correction was used if necessary. Total PANSS scores and its subscores, cortisol, IL-2, and IL-6 levels were examined by multivariate regression analyses. All statistical tests were two-tailed and were considered to be statistically significant at $p<0.05$.

RESULTS

Demographic Data

Table 1 shows characteristics of the patients and normal controls. No significant relationships between age or sex and serum IL-2, IL-6, or cortisol were noted either for the whole group, or when the normal controls and patients were examined separately. Age of onset of psychosis, duration of illness, and hospitalization did not significantly correlate with IL-2, IL-6, and cortisol levels in the patient group.

Clinical Results

Table 2 showed statistically significant differences between baseline and after 12 weeks of treatment with either risperidone or haloperidol. Both drugs improved PANSS total scores and all subscore measures ($p<0.01$–0.001). Further comparison between risperidone and haloperidol show that risperidone showed greater improvement on PANSS total score ($p=0.03$) and the general psychopathology subscore ($p=0.01$).

Changes of Serum IL-2, IL-6, and Cortisol before and after Treatment

IL-2 was detected in 72 patients (92.3%) and 26 controls (86.7%), and IL-6 in 55 patients (70.5%) and 16 controls (53.3%). IL-2 and IL-6 not detected in patients or normal control subjects were not included in the analysis.

Serum cortisol was significantly lower in healthy controls (72.2 ± 24.7 ng/ml) compared to patients at baseline

Table 2 Comparison of Scores at Baseline and Week 12 on the Total and Subscores of PANSS in Risperidone and Haloperidol Groups

	Baseline	Week 12	F[a]	df[a]	p[a]
PANSS total score					
Risperidone	82.4 ± 22.4	61.8 ± 20.6***	4.98	1,70	0.03
Haloperidol	79.2 ± 21.7	64.7 ± 16.6***			
P-subscore					
Risperidone	17.9 ± 7.5	12.9 ± 6.5***	2.15	1,70	0.14
Haloperidol	15.3 ± 7.9	11.4 ± 5.8***			
N-subscore					
Risperidone	26.6 ± 8.7	22.1 ± 8.5***	3.57	1,70	0.056
Haloperidol	28.1 ± 5.9	24.6 ± 5.5**			
G-subscore					
Risperidone	37.9 ± 11.5	26.7 ± 8.7***	6.92	1,70	0.01
Haloperidol	36.1 ± 11.8	28.6 ± 8.5***			

PASS, Positive and Negative Syndrome Scale; P-subscore, positive symptom subscore; N-subscore, negative symptoms subscore; G-subscore, general psychopathology subscore.
[a]F values and p are related to comparison between risperidone and haloperidol groups at week 12.
*Comparison between pre- and post-treatment. **$p<0.01$, ***$p<0.001$.

concentrations (103.2 ± 42.3 ng/ml; ANOVA) and at post-treatment (77.9 ± 30.7 ng/ml; $\chi^2 = 22.69$, df = 2, $p < 0.01$). Serum cortisol concentrations in SCH patients were significantly lower after treatment compared to before treatment (Wilcoxon's test, $Z = -10.45$, $p < 0.01$). Baseline levels of IL-2 in healthy controls (3.3 ± 1.4 ng/ml) were significantly lower compared to SCH subjects (9.6 ± 5.2 ng/ml) even at post-treatment (6.7 ± 4.6 ng/ml; $\chi^2 = 11.89$, df = 2, $p < 0.01$). Finally, serum IL-2 concentrations in SCH patients were significantly lower after treatment (Wilcoxon's test, $Z = -4.33$, $p < 0.01$).

Table 3 shows a significantly lower cortisol level at post-treatment in risperidone group compared to the haloperidol group ($Z = -2.46$, $p < 0.05$). In addition, no significant difference in IL-6 concentrations was noted between before and after treatment for both groups (all $p > 0.05$). Both risperidone and haloperidol significantly decreased cortisol and IL-2 levels compared to baseline (see Table 3).

Relationship among Cortisol, IL-2, and IL-6

Table 4 demonstrates a positive correlation between cortisol and IL-2 ($r = 0.36$, df = 75, $p < 0.01$) and IL-6 ($r = 0.41$, df = 75, $p < 0.01$) in the SCH group. Serum IL-2 and IL-6 ($r = 0.42$, df = 30, $p < 0.05$) and serum IL-6 and cortisol were also positively corrected in healthy controls ($r = 0.38$, $n = 30$, $p < 0.05$).

Table 3 Comparison of Serum Cortisol, IL-2 and IL-6 Levels at Baseline and Week 12 in Risperidone and Haloperidol Groups (ng/ml)

	Baseline	Week 12	Z	p
Cortisol				
Risperidone	104.2 ± 44.9	72.0 ± 31.7*	−3.27	0.002
Haloperidol	106.2 ± 41.7	83.8 ± 28.6	−2.84	0.005
IL-2				
Risperidone	9.8 ± 4.7	7.2 ± 4.9	−3.46	0.0015
Haloperidol	9.9 ± 5.2	6.1 ± 4.3	−3.64	0.0011
IL-6				
Risperidone	0.3 ± 0.5	0.23 ± 0.4	−0.24	0.68
Haloperidol	0.2 ± 0.3	0.17 ± 0.2	−0.11	0.92

Note: * indicates comparison between risperidone *vs* haloperidol, *$p = 0.014$.

Table 4 Intercorrelations between Cortisol, IL-2 and IL-6 in Patients with Schizophrenia before and after 12-Week Treatment

	COR T1	COR T2	IL-2 T1	IL-2 T2	IL-6 T1	IL-6 T2
COR T1	—					
COR T2	0.12	—				
IL-2T1	0.36**	0.16	—			
IL-2T2	0.17	0.04	0.41**	—		
IL-6T1	0.41*	−0.03	0.06	0.17	—	
IL-6T2	0.11	0.12	0.15	0.12	0.38**	—

**Correlation is significant at the 0.01 level.
Note: T1 and T2 indicate the cortisol and interleukin levels at baseline and at post-treatment.

Relation between Cortisol, IL-2, or IL-6 and Clinical Symptoms at Pre- and Post-Treatment

The results from Table 5 suggest that cortisol levels were indeed associated with negative symptomology. In addition, IL-2 levels were negatively correlated with PANSS positive subscore ($r = -0.31$, df = 71, $p < 0.01$) at pretreatment.

Relation of Cortisol and Cytokines with Clinical Outcome

Reduction in PANSS total score and its subscores after treatment determined the efficacy of antipsychotic drugs. The change of IL-2, IL-6, and of cortisol refers to the values of these parameters before treatment minus their respective values after treatment.

The results showed that there was a significant correlation between the reduction of PANSS total score and the change of IL-2 before and after treatment ($r = 0.38$, df = 65, $p < 0.01$) or cortisol levels at post-treatment ($r = -0.36$, df = 67, $p < 0.01$). Reduction in PANSS positive subscore was negatively correlated with serum IL-2 at baseline ($r = -0.36$, df = 67, $p < 0.01$). Moreover, the reduction of PANSS negative subscore was associated with decrease in serum cortisol between pre- and post-treatment ($r = 0.34$, df = 69, $p < 0.01$). No correlations were observed between the change of IL-6 before and after treatment and the reduction of the PANSS total score and its subscores (all $p > 0.05$).

A set of regression analyses was conducted in order to identify the possible contribution of the cortisol, IL-2, and IL-6 to clinical outcome (as defined by the reduction of PANSS total score). A simultaneous entry regression analysis was conducted first, with age, sex, duration of illness, and measures of cortisol, IL-2, and IL-6 at both pre- and post-treatment, as well as the changes in cortisol, IL-2, and IL-6 between pre- and post-treatment as dependents. The overall analysis was statistically significant ($R = 0.707$, $R^2 = 0.50$, $F = 3.77$, $p = 0.002$). When this analysis was repeated with a forward entry stepwise regression analysis, sex was entered into the regression equation first ($\beta = 0.45$, $t = 3.26$, $p = 0.002$), and accounted for 18.3% of the variance, followed by IL-2 levels at baseline ($\beta = -0.33$, $t = -2.56$, $p = 0.014$) and the change in cortisol between pre- and post-treatment ($\beta = 0.26$, $t = 2.04$, $p = 0.048$), which accounted for 9.5 and 5.2%, respectively.

DISCUSSION

Elevated levels of cortisol in SCH found in the present study are in agreement with some previous studies (Newcomer *et al*, 1991; Tandon *et al*, 1991; Lammers *et al*, 1995; Meltzer *et al*, 2001), but not with others (Jansen *et al*, 1998; Kaneda *et al*, 2002). Our finding of positive correlation between cortisol and negative symptoms is consistent with some previous studies (Newcomer *et al*, 1991; Tandon *et al*, 1991; Shirayama *et al*, 2002). In contrast, some other studies

Table 5 Intercorrelations between Cortisol, IL-2 and IL-6 and Clinical Variables in Patients with Schizophrenia before and after 12-Week Treatment

	COR T1	CORT2	IL-2T1	IL-2T2	IL-6T1	IL-6T2
Age	−0.15 (028)	0.13 (0.36)	0.04 (0.78)	0.06 (0.66)	0.02 (0.87)	0.02 (0.88)
Duration of illness	0.06 (0.67)	−0.05 (0.73)	−0.01 (0.95)	0.02 (0.91)	0.13 (0.34)	0.15 (0.31)
PANSS total score (T1)	0.17 (0.17)	−0.04 (0.72)	−0.19 (0.12)	−0.01 (0.93)	−0.11 (0.39)	−0.09 (0.43)
PANSS positive (T1)	0.05 (0.69)	−0.12 (0.34)	−0.31 (0.01)*	−0.08 (0.51)	−0.01 (0.93)	−0.06 (0.59)
PANSS negative (T1)	0.28 (0.02)*	0.04 (0.77)	0.03 (0.84)	0.08 (0.51)	0.07 (0.56)	0.06 (0.62)
PANSS total score (T2)	0.03 (0.78)	0.30 (0.01)*	−0.18 (0.15)	−0.08 (0.52)	0.08 (0.49)	0.07 (0.53)
PANSS positive (T2)	0.14 (0.24)	0.03 (0.84)	0.11 (0.35)	−0.01 (0.94)	0.01 (0.93)	0.01 (0.92)
PANSS negative (T2)	−0.08 (0.49)	0.34 (0.005)**	−0.12 (0.32)	−0.02 (0.89)	0.01 (0.99)	0.01 (0.93)

Note: T1 and T2 indicate the cortisol, interleukin levels, or PANSS scores at baseline and at post-treatment. The numbers in the parentheses indicate the *p*-value.

found a correlation between cortisol and positive symptoms (Kaneko *et al*, 1992; Walder *et al*, 2000). Differences in experimental methodology between studies may explain divergent studies. These include: differences in tested material (serum *vs* plasma), sampling of patients in different stages of disease progression (acute *vs* chronic or active phase *vs* remission), exposure to a variety and duration of neuroleptic treatments and different disease progression. Nevertheless, our results clearly show that cortisol is elevated in patients with SCH and this elevation is positively correlated with negative symptoms.

Some previous study found abnormalities in the cytokine system in treatment-refractory SCH patients (Lin *et al*, 1998; Maes *et al*, 2000). In our present study, we also found that levels of IL-2 and IL-6 were elevated in patients with treatment-refractory SCH, suggesting again activation of the IRS. Moreover, serum IL-2 or IL-6 and cortisol were positively correlated in our SCH population providing support for the hypothesis that hypercortisolemia observed in SCH could be induced by proinflammatory cytokines (Altamura *et al*, 1999).

Cytokine IL-2 exerts numerous effects within the immune as well as the central nervous system and is thought to serve as a humoral signal in their communication. IL-2 plays a major role in regulating HPA axis action, which may occur at different levels of regulation (Haddad *et al*, 2002). For example, Hanisch *et al* (1994) found that IL-2 caused a significant increase in ACTH levels during the later portion of the dark phase of the cycle, and plasma corticosterone concentrations were significantly elevated over almost the entire diurnal cycle (Hanisch *et al*, 1994). An additional study showed that HPA axis activation can be elicited by exogenous cytokines (IL-1, IL-6, TNF-α) when administered to rodents (Wang and Dunn, 1999). Injection of IL-6 into humans increases plasma adrenocorticotropic hormone and plasma cortisol (Bethin *et al*, 2000; Steensberg *et al*, 2003). Moreover, both the pituitary corticotrophs and adrenocortical cells express IL-6 receptors, and IL-6 increases cortisol both directly and indirectly (Bethin *et al*, 2000). Taken together, the data indicate that IL-2 and IL-6 are potent activators of the HPA axis, which provide additional evidence to support the hypothesis that hypercortisolemia in SCH may be mediated by the elevated cytokines.

Risperidone, which is more effective on negative symptoms, decreased cortisol serum levels more to a greater degree than haloperidol in the present study. Furthermore, the significant reduction of PANSS negative subscore and the change of cortisol level before and after treatment suggests that the improvement in negative symptoms is associated with the changes in serum cortisol levels. Taken together, these findings suggest that different clinical responses to atypical *vs* typical neuroleptics, paralleled with their different effects on hypercortisolism, could offer an explanation of the additional efficacy on negative symptoms for the former drugs. In addition, it is worthy of mentioning that the effects of antipsychotic drugs on cortisol levels and/or the negative symptoms may be not directly related to the changes in IL-2 or IL-6, because no significant relationships were found among these variables during treatment.

Risperidone is a relatively new atypical antipsychotic agent, with potent serotonin-5-HT2A and dopamine-D2 receptor blocking properties (Janssen *et al*, 1988). The superiority of risperidone over haloperidol on negative symptoms may be related to its antagonism of 5-HT2 in different brain areas (Wirshing *et al*, 1999; Geddes *et al*, 2000; Zhang *et al*, 2001). On the other hand, there is an increasing evidence that 5-HT promotes HPA axis activity at the hypothalamic, pituitary, and adrenal levels (Chaouloff, 1993; Dinan, 1996). Pharmacological stimulation of different 5-HT receptor subtypes as well as stress-induced 5-HT release promote activation of the HPA axis and subsequent release of CRH, ACTH, and glucocorticoids (Chaouloff, 1993). In relation to the present study, Muck-Seler *et al* (1999) showed that SCH patients have a HPA axis dyregulation and that this may be related to abnormalities in the 5-HT system (Muck-Seler *et al*, 1999). Thus, risperidone's action at 5HT2 receptors may explain its greater efficacy on negative symptoms and its ability to decrease cortisol serum levels compared to the typical neuroleptic haloperidol.

Several points concerning the reliability of measuring IL-2 levels should be noted. Many researchers are not able to detect IL-2 levels in serum which is most likely due to the assay employed. We utilized radioimmuno-assay which detects pico-gram quantities of the target protein in question. This highly sensitive assay may be the reason we were able to detect IL-2 in SCH patients and in normal

controls with significantly higher levels in patients. Another possible explanation is that there are significant differences in IL-2 genotype frequencies observed in different populations. Recent studies have shown that inheritance of polymorphic cytokine gene alleles is dramatically influenced by ethnicity (Cox *et al*, 2001; Hoffmann *et al*, 2002). Moreover, a statistically significant ethnicity-based variability in the allelic frequency and genotype inheritance patterns for IL-2 and IL-6 has been reported (Cox *et al*, 2001), suggesting that polymorphisms within these cytokine genes may be responsible for the ethnic-based differences in IL-2 or IL-6 levels. Unfortunately, there is no comparison study on the IL-2 genotype between Chinese and Caucasians. In regards to the present study, the possible effects of antipsychotic treatment before the washout period may play a role. Our patients had undergone chronic long-term treatment with antipsychotics. Studies have shown that typical and atypical antipsychotic drugs exert immunosuppressive effects on the production of cytokine (Maes *et al*, 1995; Song *et al*, 2000). IL-2 and IL-6 levels were measured after patients underwent a 2-week washout period, thus it is possible that elevated levels is a rebound effect following drug withdrawal. To answer this question, future research should examine the effects of antipsychotics on cytokine levels in naive, first-episode SCH patients.

In summary, our results suggest that dysregulation in neuro-immune-endocrine systems exist and are linked to clinical symptoms of SCH. The differential effects of typical *vs* atypical antipsychotics on the HPA axis and IRS in SCH could account, at least in part, for the better clinical outcome, especially for negative symptoms achieved in patients treated with the latter drugs compared to those receiving conventional neuroleptics.

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