Schizophrenia Research 212 (2019) 92–98

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

# The effect of minocycline on amelioration of cognitive deficits and pro-inflammatory cytokines levels in patients with schizophrenia

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ARTICLE INFO

*Article history:*
Received 31 May 2019
Received in revised form
31 July 2019
Accepted 3 August 2019
Available online 12 August 2019

*Keywords:*
Minocycline
Schizophrenia
Cognition
Interleukin-1β
Interleukin-6
Tumor necrosis factor−α

ABSTRACT

*Background:* Cognitive deficits of schizophrenia are predictors of poor function, but antipsychotic medication has limited efficacy for cognitive deficits. These deficits in learning and memory may result from activity of pro-inflammatory cytokines, which microglia produce. The microglia inhibitor minocycline might arrest this cytokine damage to the hippocampus and reverse the cognitive deficits of schizophrenia.
*Methods:* A double-blind, placebo-controlled study involved 75 patients with schizophrenia who randomly received low dose (100 mg/day) or high dose minocycline (200 mg/day) or placebo added to risperidone. MATRICS Consensus Cognitive Battery (MCCB) was used to assess the cognitive functioning, and serum levels of Interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor−α (TNF-α) were assessed.
*Results:* $\text{Minocycline}_{\text{high dose}}$ group was significantly superior to $\text{minocycline}_{\text{low dose}}$ or placebo group not only for the improvements in cognitive tests' scores as well ($P < 0.05$), but for IL-1β and IL-6 serum levels reduction ($P < 0.01$). The amelioration of cognitive deficits with minocycline correlated not only with the remission of negative symptoms, but also with the reduction in serum levels of IL-1β and IL-6.
*Conclusions:* Minocycline adjunctive treatment was effective in improving cognitive deficits of patients with schizophrenia. The beneficial effect of minocycline may be related to reducing pro-inflammatory cytokines through microglia inhibition.



## 1. Background

Schizophrenia is a severe mental disorder marked by delusions, hallucinations, negative symptoms, cognitive impairments, and can potentially become a lifetime burden for many patients (Zhang and Zhao, 2014). Currently, there is ineffective treatment for the all symptoms of schizophrenia, especially for cognitive deficits. Understanding the cause of schizophrenia helps us with effective treatments for specific biological subtypes of schizophrenia. Previous studies have proposed a “microglia hypothesis of schizophrenia” in which microglia release of pro-inflammatory cytokines mediates neuro-inflammation and brain damage in schizophrenia (Horvath and Mirnics, 2014; Fan et al., 2007; Zhao and Zhang, 2015; Monji et al., 2009; Monji et al., 2013). Immune-related disturbances and neuro-inflammation may lead to cognitive deficits in schizophrenia (Tay et al., 2017; Blank and Prinz, 2013; Fillman et al., 2013; Racki et al., 2016). Thus, finding potential neuroinflammatory biomarkers of schizophrenia has been challenging and new treatment targets is needed.

Minocycline, a second-generation tetracycline, is known to have anti-inflammatory effects and is able to inhibit microglial activation (Plane et al., 2010). Animal models of schizophrenia have reported that minocycline could alleviate behavioral changes, such as hyperlocomotion, social interaction and pre-pulse inhibition deficits (Zhang et al., 2007; Mizoguchi et al., 2008; Zhu et al., 2014a, 2014b). Some clinical trials have also shown that minocycline treatment can improve cognitive functioning in schizophrenia patients (Levkovitz et al., 2010; Liu et al., 2014; Kelly et al., 2015). Therefore, we have hypothesized that minocycline may ameliorate cognitive

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https://doi.org/10.1016/j.schres.2019.08.005
0920-9964/

deficits in schizophrenia through alleviating neuro-inflammation. To test this hypothesis we completed this double-blind, placebo-controlled study to assess the efficacy of minocycline for cognitive deficits of schizophrenia patients and to detect the serum levels of pro-inflammatory cytokines.

## 2. Methods

### 2.1. Participants

Patients aged 18 to 45 years with a diagnosis of schizophrenia (the structured clinical interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition diagnosis, SCID) were recruited from Guangzhou Baiyun Psychiatric Hospital between June 2013 and September 2014. Subjects with a higher negative subscale score than positive subscale score on the Positive and Negative Syndrome Scale (PANSS) were included, and the PANSS negative score of subjects on baseline was greater than 20 with at least one moderate negative symptom (Potkin et al., 2002; Rabany et al., 2011). The disease duration of patients was limited to 2 to 10 years. All patients had not taken antipsychotics for at least half a month before study entry. Exclusion criteria were significant inflammatory or immune conditions, minocycline allergy and pregnancy. Patients with anti-inflammatory drugs, hormones or immunosuppressant agents in the last half year were also cause for exclusion.

### 2.2. Procedures

The study was a 3-month, randomized double-blind placebo controlled comparison of adjunctive minocycline or placebo added to risperidone. The protocol was approved by the ethics committee of Guangzhou Baiyun Psychiatric Hospital.

After the screening meeting including SCID assessment, patients who signed the informed consent form were randomized to receive low dose minocycline (100 mg/day) or high dose minocycline (200 mg/day) or placebo. The dose of risperidone was able to be adjusted between 3 mg and 6 mg/day. The combined use of lorazepam and trihexyphenidyl hydrochloride was allowed to respectively treat insomnia or extrapyramidal symptoms.

### 2.3. Outcome measures

The primary outcome was the change of cognitive functioning after treatment. Cognitive evaluation was performed using eight tests from the MATRICS Consensus Cognitive Battery (MCCB) covering six cognition domains (Green and Nuechterlein, 2004): Trail Making Test (TMT), Symbol Coding Test, Verbal Fluency, the Continuous Performance Test-Identical Pairs (CPT-IP), Spatial Span, Hopkins Verbal Learning Test-Revised (HVLT-R), Brief Visuospatial Memory Test- Revised (BVMT-R) and Mazes. Investigators completed the consistency training for these scales (Kappa = 0.85).

We made follow-up visits at month 3 after starting treatment. Each enrolled patient was underwent a series of assessments both at baseline and month 3 including the Treatment Emergent Symptom Scale (TESS), physical examinations including weight, routine laboratory tests and concomitant medication. PANSS was assessed at baseline and month 1, 2, 3, while MCCB was assessed at baseline and month 3 to avoid the practice effect of cognitive function tests. The date and reason of dropping-out from the study were recorded.

Interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor−α (TNF-α) serum levels were analyzed by quantitative enzyme-linked immunosorbent assay (ELISA) at baseline and month 3. The fasting forearm venous blood samples were collected in schizophrenia patients and 30 healthy volunteers.

### 2.4. Data analysis

Statistical analysis was performed using SPSS version 22.0. Since we collected MCCB and pro-inflammatory cytokine measurements at baseline and month 3, we used the data from all randomized patients (per-protocol analysis). Continuous variables were described using summary statistics such as means and standard deviations. Categorical variables were described using frequencies and percentages. We performed baseline between-group comparisons using the analysis of variance (ANOVA) for continuous variables and Chi-square analysis for categorical variables.

The main strategy involved univariate analysis of variance for the changes of each MCCB score and pro-inflammatory cytokine serum level after treatment, using a between-group factor of drug (placebo, 100 mg and 200 mg minocycline), with baseline value as a covariate. We used the Bonferroni post hoc test to compare the specific treatments between groups. The within-dosage group comparisons were performed using the paired *t*-test for continuous variables, and the Fisher exact test for categorical variables. The relationship between the changes in cytokine serum levels and cognitive functioning was analyzed using Pearson correlation coefficients. We investigated potential response predictors associated with changes in cognitive functioning using multiple linear regression. For all analyses, a *P* value less than 0.05 (2-tailed) was used for statistical significance.

## 3. Results

### 3.1. Demographic and basic descriptive data

We randomized 75 of the 110 patients screened to minocycline$_{high\ dose}$, minocycline$_{low\ dose}$ or placebo group (25 patients on each group). 57 patients completed the whole 3-month trial (Fig. 1), and there was no difference in completion rates between groups (minocycline$_{high\ dose}$ group: n = 18, minocycline$_{low\ dose}$ group: n = 20, placebo group: n = 19). The reasons for dropout in the per-protocol population did not appear related to side effects of the medication and did not differ from the placebo dropouts. There were no statistical differences in demographic or clinical characteristics between the three groups at baseline (Table 1). The average daily dose of risperidone was not statistically different between the 3 treatment groups (Table 1).

### 3.2. Change in cognitive functioning

All cognitive tests' scores of the three treatment groups both at baseline and at month 3 were obviously worse than those of healthy volunteers ($P < 0.01$). However, patient scores improved obviously after 3-month treatment ($P < 0.01$) (Table 2).

By using ANCOVA, we found a significant difference in the improvement of the following 6 MCCB test scores between baseline and month 3: TMT ($df_1 = 2$, $df_2 = 54$, $F = 21.885$, $P = 0.000$, ES = 0.448, 95%CI: 0.234–0.579), BACS SC ($df_1 = 2$, $df_2 = 54$, $F = 4.530$, $P = 0.015$, ES = 0.144, 95%CI: 0.005–0.297), Verbal Fluency ($df_1 = 2$, $df_2 = 54$, $F = 15.434$, $P = 0.000$, ES = 0.364, 95%CI: 0.152–0.509), HVLT-R ($df_1 = 2$, $df_2 = 54$, $F = 4.543$, $P = 0.015$, ES = 0.144, 95%CI: 0.006–0.298), BVMT-R ($df_1 = 2$, $df_2 = 54$, $F = 7.340$, $P = 0.002$, ES = 0.214, 95%CI: 0.039–0.371), Maze ($df_1 = 2$, $df_2 = 54$, $F = 3.566$, $P = 0.035$, ES = 0.117, 95%CI: 0.000–0.266) (Table 3). After the Bonferroni post hoc test, the improvement of TMT and Verbal Fluency score on minocycline$_{high\ dose}$ were both significantly greater than on minocycline$_{low\ dose}$ and placebo (both $P < 0.05/3 = 0.017$) (Table 3). The improvement of HVLT-R and BVMT-R score on minocycline$_{high\ dose}$ were obviously greater than on minocycline$_{low\ dose}$ ($P = 0.017$, $P = 0.002$ respectively) (Table 3). But the changes in MCCB tests' scores on minocycline$_{low\ dose}$ were not obviously different with the changes on placebo.

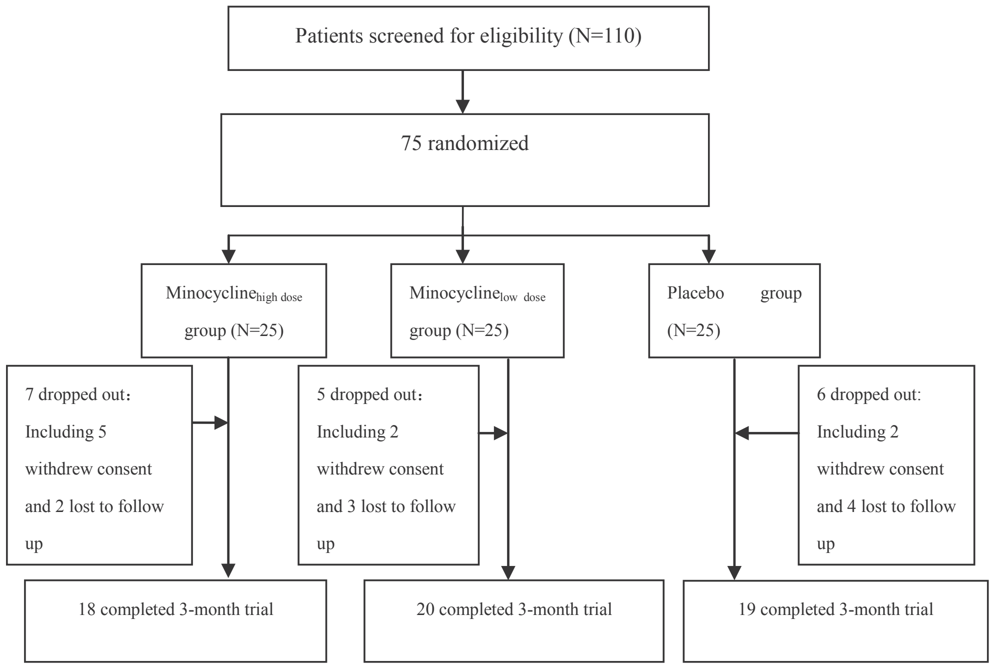


**Fig. 1.** Flowchart of participation in the study.

### 3.3. Adverse events

No serious adverse events occurred in the trial. There were no significant differences in the types and frequency of adverse events between the 3 treatment groups (Supplemental Table 1).

### 3.4. Change in cytokine serum levels

Either at baseline or at month 3, serum levels of IL-1β, IL-6 and TNF-α in each treatment group were all significantly higher than those of healthy volunteers. But the decrease in serum levels of each cytokine reached statistical significance within the 3 groups after 3-month treatment ($P < 0.01$) (Table 2).

After 3-months of treatment, the reduction on IL-1β ($df_1 = 2$, $df_2 = 54$, $F = 73.357$, $P = 0.000$, ES = 0.731, 95%CI: 0.589–0.799) and IL-6($df_1 = 2$, $df_2 = 54$, $F = 112.981$, $P = 0.000$, ES = 0.807, 95%CI: 0.700–0.856) serum levels significantly differed among the 3 treatment groups. However, the reduction of TNF-α serum levels did not reach statistical significance among these three groups ($P > 0.05$) (Table 3). After Bonferroni tests, minocycline$_{high\ dose}$ had

**Table 1**
Baseline demographic and clinical characteristics.

| Variable | Group | | | Analysis | |
|---|---|---|---|---|---|
| | Minocycline$_{low\ dose}$ group (n = 20) | Minocycline$_{high\ dose}$ group (n = 18) | Placebo group (n = 19) | | |
| | | Mean (SD) | | $F$ | $P$ |
| Age (years) | 33.60(7.73) | 34.22(6.73) | 34.68(6.43) | 0.118 | 0.889 |
| Education (years) | 11.20(3.19) | 11.17(2.98) | 11.05(3.42) | 0.011 | 0.989 |
| Duration of illness (years) | 6.39(1.87) | 6.29(1.75) | 6.52(1.74) | 0.071 | 0.931 |
| Dose of risperidone (mg/d) | 4.43(0.50) | 4.47(0.51) | 4.43(0.55) | 0.041 | 0.960 |
| PANSS[a] total score | 79.50(5.32) | 79.06(4.95) | 78.37(4.82) | 0.248 | 0.781 |
| MCCB[b] | | | | | |
| TMT (sec) | 54.61(12.15) | 56.16(14.11) | 57.30(11.96) | 0.220 | 0.803 |
| BACS SC (n) | 37.75(5.80) | 39.17(6.53) | 40.42(7.54) | 0.788 | 0.460 |
| Verbal Fluency (n) | 11.85(2.54) | 12.00(2.06) | 12.26(2.79) | 0.137 | 0.872 |
| CPT-IP total score | 1.45(0.69) | 1.22(0.55) | 1.16(0.50) | 1.338 | 0.271 |
| Spatial span (n) | 11.05(2.58) | 11.44(2.66) | 11.42(2.29) | 0.150 | 0.861 |
| HVLT-R (n) | 13.35(3.44) | 13.11(3.41) | 12.68(3.97) | 0.169 | 0.845 |
| BVMT-R (n) | 16.10(3.40) | 16.33(3.61) | 15.26(3.18) | 0.515 | 0.601 |
| Maze (sec) | 10.50(3.40) | 11.06(3.49) | 11.53(3.08) | 0.466 | 0.630 |
| IL-1β serum level (pg/ml) | 25.52(4.77) | 25.96(4.17) | 26.54(5.03) | 0.235 | 0.792 |
| IL-6 serum level (pg/ml) | 28.66(4.38) | 30.16(4.66) | 29.49(4.57) | 0.520 | 0.598 |
| TNF-α serum level (pg/ml) | 35.31(6.92) | 34.92(7.38) | 36.58(8.36) | 0.247 | 0.782 |
| | | N (%) | | $x^2$ | $P$ |
| Gender | | | | | |
| Male | 10(50.00) | 9(50.00) | 10(52.60) | 0.035 | 0.983 |
| Female | 10(50.00) | 9(50.00) | 9(47.40) | | |
| Positive psychiatric family history | 4(20.00) | 3(16.70) | 3(15.80) | 0.133 | 0.935 |

Note: The analysis of variance (ANOVA) was used for continuous variables, Chi-square analysis was used for categorical variables.
[a] The Positive and Negative Syndrome Scale.
[b] MATRICS Consensus Cognitive Battery.

**Table 2**
Treatment outcomes for 57 schizophrenia patients completed 3-month trial.

| Items | Visit time | Schizophrenia patients: Placebo group (n = 19) | Minocycline$_{low\ dose}$ group (n = 20) | Minocycline$_{high\ dose}$ group (n = 18) | Healthy volunteers (N = 30) | Between-group difference (3 treatment groups) *F* | *P* | Between-group difference (3 treatment groups and healthy volunteers) *F* | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Speed of information processing | | | | | | | | | |
| TMT (sec) | Baseline | 57.30(11.96)☆☆ | 54.61(12.15)☆☆ | 56.16(14.11)☆☆ | 25.34(6.76) | 0.220 | 0.803 | 50.851 | 0.000 |
| | Month 3 | 53.86(12.25)**☆☆ | 51.36(12.64)**☆☆ | 50.86(14.33)**☆☆ | | 23.504 | 0.000 | 37.001 | 0.000 |
| BACS SC (n) | Baseline | 40.42(7.54)☆☆ | 37.75(5.80)☆☆ | 39.17(6.53)☆☆ | 71.33(6.51) | 0.788 | 0.460 | 156.747 | 0.000 |
| | Month 3 | 43.26(8.01)**☆☆ | 40.45(6.45)**☆☆ | 42.94(7.02)**☆☆ | | 5.019 | 0.010 | 115.809 | 0.000 |
| Verbal Fluency (n) | Baseline | 12.26(2.79)☆☆ | 11.85(2.54)☆☆ | 12.00(2.06)☆☆ | 22.30(3.78) | 0.137 | 0.872 | 76.610 | 0.000 |
| | Month 3 | 13.05(3.01)**☆☆ | 12.35(2.72)**☆☆ | 13.78(2.44)**☆☆ | | 15.785 | 0.000 | 57.654 | 0.000 |
| Attention/vigilance | | | | | | | | | |
| CPT-IP total score | Baseline | 1.16(0.50)☆☆ | 1.45(0.69)☆☆ | 1.28(0.67)☆☆ | 3.17(0.65) | 1.338 | 0.271 | 63.721 | 0.000 |
| | Month 3 | 1.84(0.69)**☆☆ | 2.00(0.86)**☆☆ | 2.06(0.87)**☆☆ | | 68.041 | 0.000 | 16.753 | 0.000 |
| Working memory | | | | | | | | | |
| Spatial span (n) | Baseline | 11.42(2.29)☆☆ | 11.05(2.58)☆☆ | 11.44(2.66)☆☆ | 16.57(2.67) | 0.150 | 0.861 | 27.569 | 0.000 |
| | Month 3 | 12.26(2.64)**☆☆ | 11.90(2.75)**☆☆ | 12.39(2.85)**☆☆ | | 0.157 | 0.855 | 17.158 | 0.000 |
| Verbal learning and memory | | | | | | | | | |
| HVLT-R (n) | Baseline | 12.68(3.97)☆☆ | 13.35(3.44)☆☆ | 13.11(3.41)☆☆ | 27.70(2.38) | 0.169 | 0.845 | 134.129 | 0.000 |
| | Month 3 | 13.74(4.28)**☆☆ | 14.25(3.68)**☆☆ | 14.72(3.74)**☆☆ | | 4.939 | 0.011 | 100.009 | 0.000 |
| Visual learning and memory | | | | | | | | | |
| BVMT-R (n) | Baseline | 15.26(3.18)☆☆ | 16.10(3.40)☆☆ | 16.33(3.61)☆☆ | 28.77(3.42) | 0.515 | 0.601 | 93.917 | 0.000 |
| | Month 3 | 16.32(3.42)**☆☆ | 16.95(3.69)**☆☆ | 18.11(3.86)**☆☆ | | 7.176 | 0.002 | 70.415 | 0.000 |
| Reasoning and problem solving | | | | | | | | | |
| Maze (sec) | Baseline | 11.53(3.08)☆☆ | 10.50(3.40)☆☆ | 11.06(3.49)☆☆ | 21.07(4.00) | 0.466 | 0.630 | 52.048 | 0.000 |
| | Month 3 | 12.32(3.45)**☆☆ | 11.15(3.69)**☆☆ | 12.22(3.70)**☆☆ | | 3.876 | 0.027 | 39.649 | 0.000 |
| IL-1β (pg/ml) | Baseline | 26.54(5.03)☆☆ | 25.52(4.77)☆☆ | 25.96(4.17)☆☆ | 15.71(2.44) | 0.235 | 0.792 | 42.667 | 0.000 |
| | Month 3 | 23.39(4.97)**☆☆ | 22.18(4.85)**☆☆ | 20.12(4.50)**☆☆ | | 72.166 | 0.000 | 16.929 | 0.000 |
| IL-6 (pg/ml) | Baseline | 29.49(4.57)☆☆ | 28.66(4.38)☆☆ | 30.16(4.66)☆☆ | 17.81(2.46) | 0.520 | 0.598 | 57.419 | 0.000 |
| | Month 3 | 26.41(4.55)**☆☆ | 25.11(4.36)**☆☆ | 24.18(4.86)**☆☆ | | 109.996 | 0.000 | 23.960 | 0.000 |
| TNF-α (pg/ml) | Baseline | 36.58(8.36)☆☆ | 35.31(6.92)☆☆ | 34.92(7.38)☆☆ | 22.12(2.75) | 0.247 | 0.782 | 30.110 | 0.000 |
| | Month 3 | 33.09(8.53)**☆☆ | 31.64(7.42)**☆☆ | 31.51(7.22)**☆☆ | | 0.085 | 0.918 | 15.834 | 0.000 |

Note: Within-group differences were examined using paired T-test. Between-group differences were performed using ANOVA. The corresponding baseline score was used as covariate.
** For between each time point and baseline, $P < 0.01$.
☆☆ Compared with healthy volunteers, $P < 0.01$.

significant effect on the reduction of IL-1β and IL-6 serum levels compared with both minocycline$_{low\ dose}$ and placebo (all $P < 0.017$). But compared with placebo, minocycline$_{low\ dose}$ did not have significant effect on the change of IL-1β and IL-6 serum levels (Table 3).

### 3.5. Relationship between cognitive deficits and pro-inflammatory cytokines, clinical symptoms

Pearson correlation coefficients was used to analyze the relationship between cytokine serum levels and cognitive functioning in schizophrenia patients, with the duration of illness and the dosage of risperidone as covariates. The change in the MCCB scores of completer patients in the three treatment groups showed that no outliers were driving the correlations.

For the placebo group, the increase in Verbal Fluency, Spatial span and BVMT-R scores showed significant negative correlations with the decrease of IL-1β serum levels ($r = -0.492$, df = 15, $P = 0.045$; $r = -0.514$, df = 15, $P = 0.035$; $r = -0.556$, df = 15, $P = 0.020$), but no significant correlations with either IL-6 or TNF-α serum levels (Table 4).

For the minocycline$_{low\ dose}$ group, the increase in HVLT-R ($r = -0.727$, df = 16, $P = 0.001$) and Maze ($r = -0.592$, df = 16, $P = 0.010$) scores showed significant negative correlations with the decrease of IL-6 serum levels (Table 4).

For the minocycline$_{high\ dose}$ group, the decrease in TMT score showed significant positive correlations with the decrease in IL-1β ($r = 0.587$, df = 14, $P = 0.017$) and IL-6 serum levels ($r = 0.594$, df = 14, $P = 0.015$). The increase in five MCCB scores (Verbal Fluency, CPT-IP, Spatial span, HVLT-R, BVMT-R) all showed significant negative correlations with the decreases of IL-1β and IL-6 serum levels ($P < 0.05$ or $P < 0.01$). The increase in Maze score showed a significant negative correlation with the decrease of IL-6 serum levels ($P < 0.05$) (Table 4) (Supplemental Fig. 1).

Since cognitive deficits in schizophrenia are closely related to negative symptoms, we used stepwise multiple linear regression to explore the association of cognitive deficits with cytokines and clinical symptoms in the minocycline $_{high\ dose}$ group. The dependent variables were the changes in the various components of MCCB scores from baseline to month 3. The independent variables were the changes of serum cytokine levels, the PANSS negative score and SANS score after treatment. We found that the decrease in TMT score was significantly associated with the decrease of IL-1β serum levels (beta = 0.660, $t = 3.059$, $P = 0.007$), while the increase in Verbal Fluency and Spatial span scores were significantly associated with the decrease of IL-1β serum levels (beta = −0.422, $t = -3.493$, $P = 0.003$; beta = −0.353, $t = -3.868$, $P = 0.001$ respectively). The increases in four MCCB scores (BACS SC, CPT-IP, BVMT-R, Maze) were significantly associated with the decrease of IL-6 serum levels (beta = −0.783, $t = -2.619$, $P = 0.019$; beta = −0.368, $t = -3.673$,

**Table 3**
The difference between baseline and end point of all treatment outcomes.

| Assessment levels | Schizophrenia patients | | | ANCOVA[a] | | | | Minocycline$_{high\ dose}$ VS Minocycline$_{low\ dose}$ | Minocycline$_{high\ dose}$ VS Placebo | Minocycline$_{low\ dose}$ VS Placebo |
|---|---|---|---|---|---|---|---|---|---|---|
| | Placebo group (n = 19) | Minocycline$_{low\ dose}$ group (n = 20) | Minocycline$_{high\ dose}$ group (n = 18) | F | P | ES | 95%CI | P | P | P |
| TMT (sec) | −3.44 (0.24) | −3.25 (0.23) | −5.31 (0.25) | 21.885 | 0.000 | 0.448 | 0.234 to 0.579 | 0.000 | 0.000 | 1.000 |
| BACS SC (n) | 2.84 (0.27) | 2.70 (0.27) | 3.78 (0.28) | 4.530 | 0.015 | 0.144 | 0.005 to 0.297 | 0.021 | 0.060 | 1.000 |
| Verbal Fluency (n) | 0.79 (0.17) | 0.50 (0.17) | 1.78 (0.17) | 15.434 | 0.000 | 0.364 | 0.152 to 0.509 | 0.000 | 0.000 | 0.675 |
| CPT-IP total | 0.68 (0.12) | 0.55 (0.12) | 0.83 (0.13) | 1.320 | 0.276 | 0.047 | 0.000 to 0.168 | 0.330 | 1.000 | 1.000 |
| Spatial span (n) | 0.84 (0.13) | 0.85 (0.13) | 0.94 (0.14) | 0.179 | 0.836 | 0.007 | 0.000 to 0.067 | 1.000 | 1.000 | 1.000 |
| HVLT-R (n) | 1.05 (0.17) | 0.90 (0.17) | 1.61 (0.18) | 4.543 | 0.015 | 0.144 | 0.006 to 0.298 | 0.017 | 0.088 | 1.000 |
| BVMT-R (n) | 1.05 (0.18) | 0.85 (0.17) | 1.78 (0.18) | 7.340 | 0.002 | 0.214 | 0.039 to 0.371 | 0.002 | 0.019 | 1.000 |
| Maze (sec) | 0.79 (0.14) | 0.65 (0.14) | 1.17 (0.14) | 3.566 | 0.035 | 0.117 | 0.000 to 0.266 | 0.036 | 0.199 | 1.000 |
| IL-1β (pg/ml) | −3.15 (0.17) | −3.34 (0.17) | −5.84 (0.18) | 73.357 | 0.000 | 0.731 | 0.589 to 0.799 | 0.000 | 0.000 | 1.000 |
| IL-6 (pg/ml) | −3.08 (0.14) | −3.55 (0.14) | −5.97 (0.15) | 112.981 | 0.000 | 0.807 | 0.700 to 0.856 | 0.000 | 0.000 | 0.071 |
| TNF-α (pg/ml) | −3.50 (0.44) | −3.67 (0.43) | −3.41 (0.45) | 0.087 | 0.917 | 0.003 | 0.000 to 0.060 | 1.000 | 1.000 | 1.000 |

Note: Since there were 3 comparisons among 3 treatment groups, the Bonferroni-adjusted significance level was set at $P = 0.05/3 = 0.017$.
[a] Adjusted P values.

$P = 0.002$; beta = −0.797, $t = -4.053$, $P = 0.001$; beta = −0.407, $t = -2.601$, $P = 0.019$ respectively). Finally, the increase in HVLT-R was significantly associated with the decrease of IL-6 serum levels (beta = −0.465, $t = -2.374$, $P = 0.031$) and with the reduction of PANSS negative score (beta = −0.303, $t = -2.351$, $P = 0.033$).

## 4. Discussion

In this study, we previously found that negative symptoms of schizophrenia patients obviously improved in the minocycline$_{high\ dose}$ group and that these clinical symptom improvements were correlated with decrease in pro-inflammatory cytokines (Zhang et al., 2018). These results have been published (Zhang et al., 2018). In our study, the cognitive functioning of patients with schizophrenia was evaluated at baseline and month 3. Thus, we mainly showed that cognitive functioning significantly improved among the three treatment groups after 3-months of minocycline adjunctive treatment in this report. The speed of information processing (TMT and Verbal Fluency score) improved greater on minocycline$_{high\ dose}$ than on minocycline$_{low\ dose}$ or placebo. This amelioration of cognitive deficits with minocycline correlated not only with the reduction in serum levels of IL-1β and IL-6, but also with the remission of negative symptoms.

To date, the growing evidence shows that pro-inflammatory cytokines released by abnormal activation of microglia play an important role in the etiology and cognitive impairment of schizophrenia. Some studies suggest that modulators of microglial activation (such as minocycline) may have effects on schizophrenia (Monji et al., 2009; Monji et al., 2013; Blank and Prinz, 2013; Racki et al., 2016; Tay et al., 2017). It has been found that cytokine signaling deficiency in CX3CR1 knockout mice, microglial-BDNF deletion or microglial depletion each resulted in cognitive impairment (Rogers et al., 2011; Parkhurst et al., 2013).

Our findings are consistent with some previous studies of minocycline in animals and schizophrenic patients. Zhu et al. found that minocycline was able to ameliorate behavioral deficits (social interaction and pre-pulse inhibition deficits) and inhibit the activated microglia in adult rats injected with Granulocyte-Macrophage Colony-Stimulating Factor (Zhu et al., 2014b). Several clinical studies of minocycline have improved cognitive deficits in patients

**Table 4**
The correlation between the change in cytokine serum levels and cognitive functioning.

| Symptoms index | Placebo group (N = 19) | | | Minocycline$_{low\ dose}$ group (N = 20) | | | Minocycline$_{high\ dose}$ group (N = 18) | | |
|---|---|---|---|---|---|---|---|---|---|
| | IL-1β | IL-6 | TNF-α | IL-1β | IL-6 | TNF-α | IL-1β | IL-6 | TNF-α |
| Change in TMT | 0.353 | 0.149 | 0.011 | −0.180 | 0.228 | 0.153 | 0.587* | 0.594* | 0.449 |
| Change in BACS SC | −0.362 | −0.315 | −0.039 | −0.226 | −0.323 | −0.126 | −0.495 | −0.492 | −0.420 |
| Change in verbal fluency | −0.492* | −0.148 | −0.314 | −0.022 | −0.324 | −0.137 | −0.622** | −0.545* | 0.084 |
| Change in CPT-IP | −0.469 | −0.316 | −0.093 | −0.157 | −0.168 | −0.263 | −0.540* | −0.625** | −0.138 |
| Change in spatial span | −0.514* | −0.437 | −0.156 | −0.461 | −0.727** | −0.063 | −0.640** | −0.613* | −0.241 |
| Change in HVLT-R | −0.469 | −0.421 | −0.024 | −0.253 | −0.357 | −0.017 | −0.645** | −0.654** | −0.161 |
| Change in BVMT-R | −0.556* | −0.248 | −0.382 | −0.320 | −0.293 | −0.068 | −0.682** | −0.728** | −0.273 |
| Change in maze | −0.472 | −0.447 | −0.100 | −0.388 | −0.592** | −0.236 | −0.479 | −0.530* | −0.443 |

** $P < 0.01$.
* $P < 0.05$.

with schizophrenia. However, these previous studies used different assessment tools for cognition and included different subtype of schizophrenia. Levkovitz et al. found that minocycline 200 mg/day for half a year improved executive function as measured with CANTAB, but only 13 of the 54 early stage schizophrenia patients completed the trial (Levkovitz et al., 2010). Kelly et al. carried out a 10 week study of minocycline 200 mg/day with clozepine treating 52 patients with chronic schizophrenia and assessed cognition with the MCCB. They showed that minocycline improved working memory (Kelly et al., 2015). Our research group in an earlier study showed that minocycline addition treatment for 16 weeks improved attention in 92 schizophrenia patients, who had stabilized their antipsychotic dosing and had minimal cognitive deficits (Liu et al., 2014). Chaudhry et al. did not found significant difference in cognitive function between patients receiving minocycline and placebo (Chaudhry et al., 2012). This study included patients who often had few cognitive deficits and different types of psychotic diseases, and it had no restrictions on the use of antipsychotic drugs (Chaudhry et al., 2012). To reduce the impact of pre-baseline antipsychotic medication stabilization and increase the likelihood of baseline cognitive deficits, our study included patients who had mainly negative symptoms and who had not taken antipsychotics in 2 weeks before enrollment. We also examined a lower minocycline dose (100 mg) than previous studies and showed that this dose was ineffective. High dose minocycline (200 mg) for 3-months significantly improved cognitive functioning, more than not only placebo, but also minocycline$_{\text{low dose}}$. Similarly, low dose minocycline did not significantly change MCCB. Thus, we replicated minocycline's efficacy for cognitive deficits in schizophrenia and suggest that 200 mg may be the minimal effective dose.

Numerous studies have indicated that microglia, responsible for much of the cortex's pro-inflammatory cytokine production, are important for learning and memory in the hippocampus and play a significant immunological role in schizophrenia (Williamson et al., 2011; Tambuyzer et al., 2009; Mansur et al., 2012). We extended this association to show that the improvement of cognitive function with minocycline's anti-cytokine activity significantly correlated with the reduction of negative symptoms, but not with the change of positive symptoms in humans.

Similar to previous studies, we found that patients with schizophrenia had significantly elevated levels of the three pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and that minocycline in risperidone treated patients significantly reduced these levels (Potvin et al., 2008; Fillman et al., 2013; Miller et al., 2011; Drexhage et al., 2011). Furthermore, the reduction of IL-1β and IL-6 serum levels positively correlated with improvement in cognitive functioning. These findings suggest that neuro-inflammation due to abnormal microglial activation can lead to cognitive impairments in schizophrenia (Miller et al., 2011; Drexhage et al., 2011; Hinwood et al., 2012; Wohleb et al., 2011; Bernstein et al., 2014). Two investigators have shown more specific and potentially causative connections between these cytokines and hippocampal dependent learning. Williamson et al. found that microglia are the only source of IL-1β responding to hippocampus-dependent learning and that the activity of IL-1β within hippocampus can be obviously modulated by early life infections (Williamson et al., 2011). Hein et al. found that over-expression of IL-1β in mouse hippocampus led to long-term contextual and spatial memory impairment (Hein et al., 2010).

Although previous clinical studies using minocycline in schizophrenia did not report the correlation between negative symptoms and cognitive changes, negative symptoms may mediate the relationship between cognition and functional outcome in schizophrenia (Lin et al., 2013; Ventura et al., 2009; Ahmed et al., 2018). Our previous study found that the remission of negative symptoms with minocycline obviously correlated with the decrease of IL-1β and IL-6 serum levels (Zhang et al., 2018). Thus, neuro-inflammation may be a common pathophysiological mechanism for cognitive deficits and negative symptoms in schizophrenia, with elevated IL-1β and IL-6 levels as neuro-inflammatory biomarkers of these two complications of this disease.

This study has several limitations. First, the duration of the study was short, and we did not follow up the changes in cognitive functioning and pro-inflammatory cytokines levels beyond 3 months. The duration of these positive effects from minocycline is unclear. Second, we measured only serum levels of three cytokines, which may not reflect central inflammation (Beumer et al., 2012). Furthermore, several studies have not found elevated cytokines in schizophrenia (Potvin et al., 2008; Miller et al., 2011; Goldsmith et al., 2016). Third, our sample size is small and from a single ethnicity. Larger samples from different ethnicities will be needed to confirm our findings. Fourth, chronic smoking can deteriorate the cognitive deficits in patients with schizophrenia (Zhang et al., 2013; Reed et al., 2016), but we did not gather the information about smoking status and body mass index of patients.

Despite these limitations, the current study suggests that the addition of minocycline to antipsychotics is a potential treatment to improve the cognitive deficits of schizophrenia by inhibiting neuroinflammation. Further clinical trials of drugs targeting microglia activity might verify whether inhibiting microglial function can benefit the cognitive deficits in schizophrenia.

## Contributors

Jingping Zhao designed the study and wrote the study protocol. Lulu Zhang, Hongbo Zheng and Jingping Zhao acquired the data and performed the statistical analyses. Lulu Zhang, Jingping Zhao, Hongbo Zheng and Xiang-Yang Zhang interpreted the data and drafted the initial manuscript. All authors contributed in the final drafting and critically revised the manuscript.

## Role of the funding source

This study was supported by grants from the National Key Research and Development Program (Grant No.2016YFC1306900), the National Natural Science Foundation of China (Grant Nos. 81571310, 81630033, 81471363), the Natural Science Foundation of Guangdong Province (Grant No. 2017A030313809).

## Declaration of competing interest

The authors have no conflicts to disclose.

## Acknowledgements

None.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2019.08.005.

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